Dynamics and Kinetics of Singlet Oxygen Mediated Oxidation of Methionine in the Gas Phase, Hydrated Clusters and Solution

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Dynamics and Kinetics of Singlet Oxygen Mediated Oxidation of Methionine
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by

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Abstract

Dynamics and Kinetics of Singlet Oxygen Mediated Oxidation of Methionine in the Gas Phase, Hydrated Clusters and Solution

by

Fangwei Liu

Advisor: Dr. Jianbo Liu

The reaction between methionine (Met) and electronically excited singlet molecular oxygen ($O_2[a^1\Delta_g]$) has been investigated in a systematic fashion, using a home-built electrospray ionization (ESI) guided-ion-beam tandem mass spectrometer (MS). The study started from probing the reaction dynamics between the isolated protonated/deprotonated methionine ions with $^1O_2$ in the gas phase, transited through the same systems micro-solvated with explicit water molecules in gaseous hydrated clusters, and concluded with real-time methionine oxidation kinetics determination in aqueous solution. The reaction products, cross sections, and collision energy dependence were measured by ESI-MS. Density functional theory (DFT) calculations, Rice-Ramsperger-Kassel-Marcus (RRKM) statistical modeling and direct dynamics simulations were carried out to construct the potential energy surface (PES) along their reaction coordinates (including reactants, intermediate complexes, transition states, and products), analyze thermodynamics and energy barriers, as well as provide insight into the different types of stabilization of reactive species upon oxidation.

In project 1, in order to elucidate the charge effects on the reaction mechanism, the reaction were explored between $^1O_2$ and gas-phase dehydrated methionine in both protonated (MetH$^+$)
and deprotonated ([Met - H]) ionization states. For the reaction of MetH$^+$ + $^1$O$_2$, the product channel corresponds to generation of hydrogen peroxide via transfer of two hydrogen atoms from MetH$^+$ to singlet oxygen. The reaction is mediated by a precursor and/or hydroperoxide intermediate, and is sharply orientation-dependent. The reaction cross section shows strong inhibition by collision energy. No oxidation products were observed in the reaction of [Met - H]$^-$ + $^1$O$_2$, albeit the reaction is mediated by similar hydroperoxides. Due to the high energy barriers in the product exit channels, these nascent hydroperoxides cannot evolve to stable end products at the collision energy range in the present study, but decayed back to reactants.

Project 2 explored the reaction between $^1$O$_2$ and hydrated protonated/deprotonated methionine clusters (MetH$^+(\text{H}_2\text{O})_{1,2}$/[Met - H]$^-(\text{H}_2\text{O})_{1,2}$), aiming at probing the effects of charge and hydration states on the reaction mechanism, as well as mimicking the micro-solvation environment in biological systems. For the reaction of MetH$^+(\text{H}_2\text{O})_{1,2}$ + $^1$O$_2$, besides producing hydroperoxides (and their hydrates), an H$_2$O$_2$ elimination mechanism was observed. This observation indicates a transition from the gas-phase oxidation pathways to solution-phase reactions. In contrast to the non-reactivity of its dehydrated counterpart, [Met - H]$^-$ becomes oxidizable once it is hydrated by water(s); hydroperoxides and their hydrated species were captured as the oxidation products in the reactions of [Met - H]$^-(\text{H}_2\text{O})_{1,2}$ + $^1$O$_2$.

In the last project, a solution-phase reaction setup, which couples the $^1$O$_2$ generation and detection system to our ESI-MS, was developed. This on-line apparatus minimizes the sample transfer time between reaction and mass spectrometry measurements. It enables us to identify the oxidation products of Met in different pH solutions, and follow real-time reaction profiles for determining their reaction rates. Met-O is the dominant oxidation product in acidic and neutral solutions, whereas [Met - H]$^-$-O and dimeric product [Met - H]$^-$-O-Met dominate in basic
solution despite with lower reaction rates. The calculated reaction rate constants are $6 \times 10^9 \text{ M}^{-2} \cdot \text{s}^{-1}$ in acidic solution and $2 \times 10^9 \text{ M}^{-2} \cdot \text{s}^{-1}$ in basic solution, respectively.
Dedicated to the person I once was from 2007 to 2014
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Chapter 1

Background and Introduction: Singlet Oxygen ($^{1}\text{O}_2$)-Mediated Methionine Oxidation

1.1 The importance of $^{1}\text{O}_2$-mediated methionine oxidation

Molecular oxygen possesses a unique electronic configuration with a singlet excited state ($^{1}\text{O}_2[a^1\Delta_g]$), the lowest electronically excited state, commonly called singlet oxygen, lying above its triplet ground state ($^{3}\text{O}_2[X^3\Sigma_g^-]$). Singlet oxygen may be produced in biological systems via energy transfer to the ground-state $^{3}\text{O}_2$ from protein-bound or other chromophores on exposure to UV and visible light (i.e., photosensitization\(^1\)), or by a range of enzymatic and nonenzymatic reactions including those mediated by heme proteins, lipoxygenases, activated leukocytes, and radical termination reactions\(^2\). Despite its apparent simplicity, $^{1}\text{O}_2$ has a characteristic chemistry in which molecules are oxygenated setting it apart from $^{3}\text{O}_2$ which,\(^3\) because of two unpaired parallel-spin electrons, does not react with most molecules unless activated by extra energy.

$^{1}\text{O}_2$-mediated oxidation of biomolecules is a biological relevant process associates with cell death, biological aging and disease (e.g., cataract and some skin cancers),\(^4\) photodynamic therapy of cancers\(^5\) (destruction of the affected cells in the treatment of malignancies by $^{1}\text{O}_2$),\(^6\) and photochemical transformations of biological species in the atmosphere.\(^7\) Because of these biological and photochemical implications in the atmosphere, there has been considerable interest in elucidation of these reactions. It has been established that proteins are a major target for $^{1}\text{O}_2$-mediated oxidative damage in living bodies, with damage occurring preferentially at tryptophan (Trp), histidine (His), tyrosine (Tyr), methionine (Met) and cysteine (Cys) residues
because these residues have electron-rich side chains and thus are favored by electrophilic oxidizers.2,8

Methionine is structurally important in yeast enolase, lysozyme, ribonuclease A and phosphoglucomutase, etc9. Oxidation of Met residues in proteins produces MetO via addition of an oxygen atom to its sulfur atom.10 Under extremely strong oxidative conditions MetO can be irreversibly oxidized to sulfone (MetO$_2$), despite the low extent.8a The oxidation process of Met often causes proteins to lose function via conformational changes and unfolding,11 and thus is related to several pathophysiological conditions such as cancer,8a aging,12 and neurodegenerative diseases. In addition, the oxidation process of methionine has received particular attention also because of its possible role in the biological inactivation of several hormones (e.g., human growth hormone, corticotrophin, and parathyroid hormone), in the pathogenesis of several diseases (e.g., cataract), and in implication in stabilization difficulties involving pharmaceutically relevant proteins (e.g., interleukin 2 and relaxin). It is recently established that Met residues in proteins form a hydrophobic bond between their sulfur atoms and the aromatic rings of other amino residues.13 These sulfur-ring bonds could stabilize the protein structure. In contrast, the oxidation product MetO is found to be hydrophilic. Consequently, the normal 3-dimensional folding of proteins will dissociate under alteration from Met to MetO by removing the hydrophobic bond.12-13

Unlike the oxidation of other amino acid residues, a characteristic feature of Met oxidation is that cells develop a counteract process to reduce MetO back to Met. This progress is catalyzed by enzymes,14 known as methionine sulfoxide reductases (Msrs).14-15 Two types of Msrs have been identified in organism and found to stereo-selectively reduce MetO, while MsrA specifically reduces S-MetO and MsrB reduces R-MetO only. This type of thioredoxin-
dependent reduction of methionine is an evolutionary response to oxygen-induced damage in the Earth’s atmosphere and in more localized environments.\textsuperscript{16} Consequently, oxidation of methionine acts as antioxidant pool. Surface exposed Met residues can sometimes act as endogenous antioxidants or "molecular bodyguards" to protect other buried residues important to the functional of proteins under oxidative stress.\textsuperscript{17} The change in the levels of oxidized methionine residues in proteins may reflect an increase of $^1\text{O}_2$ (and other reactive oxygen species) generation, decrease of oxidant scavengers, or loss of Msrs' activities and other reducing equivalents involved. Interestingly, reversible Met oxidation process is identified as trigger for the function of two proteins,\textsuperscript{18} i.e., kinase CaMKII and the transcription factor HypT. For these reasons, methionine oxidation has been discussed most commonly by biologists and biochemists along with the photodynamic therapy on living bodies and in particular on proteins and enzymes.

It is also worth noting that amino acids (free and combined forms) are ubiquitous in tropospheric particles and depositions,\textsuperscript{19} and undergo transformations due to photochemically formed reactive oxygen species.\textsuperscript{7, 20} Because methionine can be quantitatively oxidized by $^1\text{O}_2$ (it is a pure $^1\text{O}_2$ chemical quencher) and other reactive oxygen species, the ratio of oxidized methionine/methionine can be used as a chemical marker for the transport and age of atmospheric particles and drops.\textsuperscript{7} In addition, it was reported that oxidation of methionine results in nucleation and formation of particles, which accounts for a new route for aerosol production over remote marine areas.\textsuperscript{19d} These facts demonstrate the significance of methionine oxidation on atmospheric chemistry, in addition to biological milieu.

All of these have stimulated particular interests in characterizing the reaction intermediates and product, and in interpreting the reaction mechanism of methionine oxidation.\textsuperscript{21}

1.2 Findings and problems associated with Met photooxidation experiments
Previous research on the oxidation of methionine has been mainly conducted in solution using photosensitization methods (often referred to as "photooxidation"), where $^1{\text{O}}_2$ was generated upon exposure to the ultraviolet or visible light in the presence of sensitizers (e.g., methylene blue, rose bengal, and porphyrin derivatives). The mechanisms responsible for photosensitized oxidations can be classified into two categories, referred to as Type I and Type II, respectively. Both types start with the process during which sensitizers absorb light, and are pumped to electronically excited singlet states, \( \text{i.e.,} \)

\[
^1\text{sensitizer} \xrightarrow{\text{hv}} ^1\text{sensitizer}^* 
\]

In the type I mechanism, excited singlet-state sensitizers undergo electron transfer with other molecules and generate radicals, \( \text{i.e.,} \)

\[
^1\text{sensitizer}^* + A \rightarrow A\cdot \text{ (radical)}
\]

In the type II mechanism, excited singlet-state sensitizers undergo intersystem crossing to a triplet state, (which has a longer lifetime than the singlet state), and singlet oxygen is generated via energy transfer between triplet-state sensitizer and triplet ground-state oxygen, \( \text{i.e.,} \)

\[
^1\text{sensitizer} \xrightarrow{\text{ISC}} ^3\text{sensitizer}^* 
\]

\[
^3\text{sensitizer} + ^3\text{O}_2 \rightarrow ^1\text{sensitizer}^* + ^1\text{O}_2
\]

The photooxidation results of methionine can be rationalized in terms of three different mechanisms as shown in Figure 1.1, all involving a common persulfoxide intermediate but with different stoichiometry and final products. At pH below 6, one methionine reacts with one $^1\text{O}_2$ to produce persulfoxide intermediate, which subsequently traps another methionine to produce two methionine sulfoxide molecules. At pH above 9, the sulfur atom of the persulfoxide intermediate is attacked by OH\(^-\), which generates one sulfoxide and one H\(_2\)O\(_2\). However, as the pH approaches the pK\(_a\) of the ammonium group (pK\(_a\) = 9.2), the concentration of free amine
increases, which enables the free amino group to be basic enough to attack sulfur atom in the persulfoxide intermediate. Consequently, a third process emerges and competes with the two described above. As illustrated in Figure 1.1, the third process produces S,N-heterocyclic dehydromethionine and H₂O₂.

Figure 1.1 Photooxidation paths of methionine in solution: (a) OH⁻ displacement, (b) internal displacement, and (c) formation of two sulfoxide molecules.⁹

Kinetic studies on Met oxidation by ROS, such as H₂O₂,⁸a HOCl,²² chloramines,²³ HO⁻,²⁴ and ¹⁰₂,²⁵ have also been investigated in solution, but reaction rate constants (kᵣ) for ¹⁰₂-mediated Met oxidation were reported²⁵ while ¹⁰₂ was generated via photosensitization²⁶ Consequently, unambiguous determination of reaction kinetics for ¹⁰₂-mediated reactions was often challenged by competition between radical- and ¹⁰₂-mediated reactions),⁶,²⁷ and physical quenching of ¹⁰₂ by sensitizers. As a result, it is often difficult to explicitly extract kᵣ from quenching constant (kₒ) and total reaction constant (kᵣ).
In sum, photooxidation of methionine shows complex features with several competing pathways and multiple products,\textsuperscript{2, 3c, 8-9, 28} that are easily varied by many factors (e.g., pH, oxygen concentration, solvent composition, type of sensitizers, buffer ions). This is partly due to the fact that both type I (free radical-mediated)\textsuperscript{29} and type II (\ce{^1O_2}-mediated)\textsuperscript{3d} mechanisms might exist in photooxidation and simultaneously contribute to the reaction. Both radicals and singlet oxygen could attack methionine, causing oxidative damage. Consequently, despite a few decades of studies, several fundamental aspects of methionine oxidation including products, stoichiometry, and intermediates involved have yet to be completely elucidated. To circumvent the problems associated with the solution-phase photooxidation experiments, oxidation experiments were reported using heterogeneous photosensitizers. For examples, sensitizers were immobilized on glass beads,\textsuperscript{30} or absorbed onto porous glass,\textsuperscript{30c, 30d} so that free sensitizers did not present in solution and singlet oxygen was delivered through space; accordingly, the oxidation of methionine proceeded predominantly via Type II mechanism.\textsuperscript{9}

1.3 The unique features of reaction dynamics and kinetics in our study

1.3.1 Generation of "Clean" \textsuperscript{1}O$_2$

As illustrated above, solution-phase reaction also depends on other various coupled experimental parameters such as solvent composition, oxygen concentration, buffer ions, and type of sensitizers. These factors complicate the system, and make it difficult to characterize the reaction intermediates and to interpret the reaction mechanism. This becomes the major motivation for the present thesis work. To avoid the complexities and interference arising from solution-phase photooxidation experiments and simplify the interpretation of Met oxidation mechanism, in our study \textsuperscript{1}O$_2$ was produced in the gas phase. In our previous experiment, \textsuperscript{1}O$_2$(a\textsuperscript{1}Δ$_g$) was generated by microwave discharge\textsuperscript{31} in a mixture of O$_2$/Ar, and
the $^1$O$_2$ yield was estimated using the specific energy deposition per molecule in the discharge. A microwave discharge of oxygen not only produces $^1$O$_2$, but also O and O$_3$ as well. To adequately remove O atoms through their recombination, we had to use mercuric oxide coating inside the discharge tubing. However, there are environmental restrictions on mercury use. Besides O atoms, O$_3$ (< 1%) may react to biomolecules and cause uncertainties on cross section measurements. In the present work, we have adopted a chemical $^1$O$_2$ (a$^1\Delta_g$) generator, using the reaction of

$$\text{H}_2\text{O}_2 + \text{Cl}_2 + 2\text{KOH} \rightarrow \text{O}_2 (a^1\Delta_g)/\text{O}_2(X^3\Sigma_g^-) + 2\text{KCl} + 2\text{H}_2\text{O}$$

This chemical $^1$O$_2$ generation technique has been used for creating oxygen-iodine lasers and was introduced to ion-molecule reactions with $^1$O$_2$ by Viggiano and co-workers. We adapted a similar procedure used by Viggiano et al. This chemical $^1$O$_2$ generation/detection system is capable of generating a high $^1$O$_2$ yield, without O/O$_3$ contaminants. By combining with the electrospray ionization (ESI) guided-ion beam scattering methods, our group has successfully investigated the gas-phase reaction dynamics of all five oxidizable amino acids mentioned above in both protonated and deprotonated forms with singlet oxygen. In conjunction with extensive computational simulations, we were able to learn the insight of the reaction between $^1$O$_2$ and their hydrated counterparts, and eventually unraveled the reaction kinetics and mechanism in detail.

1.3.2 **Extract the intrinsic reactivity of methionine in the gas phase**

Compared to $^1$O$_2$ chemistry in solution, much less is known about the ion-chemistry of $^1$O$_2$ in the gas phase. To the best of our knowledge, there are only a few ion-molecule reaction studies involving $^1$O$_2$, mostly concentrating on the reactions of small negative ions with $^1$O$_2$ using a flow tube by Viggiano and co-workers. Regarding the oxidation study of Met and
other amino acids, much less has been done for these systems in the gas phase. However, gas-phase experiments are no less important considering their capability on providing detailed pictures of reactions and their environmental and atmospheric implications.

One advantage of investigating biomolecules in the gas phase is that it allows one to observe single molecules separated from bulk solution environments. In this way, intrinsic reactivity of molecules can be distinguished from solvent effects and counter-ion effects, providing a basis for understanding their photooxidation mechanisms. In this thesis the first system I reported is the gas-phase reaction dynamics of $^1$O$_2$-mediated oxidation of bare Met in both protonated and deprotonated forms, using ion-beam-scattering methods$^{37}$ and electrospray-ionization mass spectrometry (ESI MS)$^{35b}$. By combining gas-phase experiments, statistical modeling and molecule dynamics simulations, we were able to unravel oxidation mechanisms and dynamics of various gas-phase "bare" amino acids ions.

It is worth noting that many groups are active in studying ion-molecule reactions of amino acids and small peptides, including using ion-molecule reactions as the probes for gas-phase structures. One useful feature of ESI is that it may allow biomolecules to retain their native-like structures when transferred from solution to the gas phase.$^{38}$ This makes the native conformations of biomolecules at least metastable in the gas phase.$^{39}$ As suggested by Bowers et al.,$^{40}$ the dielectric constant of the vacuum ($\varepsilon_{\text{vacuum}} = 1$) is close to that of the local environment of biomembranes ($\varepsilon_{\text{peptide/protein}} = 2-4$, and $\varepsilon_{\text{lipid}} = 2$); thus the gas phase resembles a biomembrane$^{41}$ and provides a simplified, yet not unrealistic, model of the membrane environment.$^{5b}$ The results obtained from the gas-phase study may then be extrapolated to a more realistic, condensed-phase in biological system.
1.3.3 Bridging the chemistry between the gas phase and solution: Gas-phase hydrates and the role of individual water(s)

An obvious question for the gas-phase experiments of protonated/deprotonated amino acids with $^1\text{O}_2$ is how to make these reactions closely resemble photooxidation reactions in biological systems where amino acids are solvated. Because amino acids ions are able to form hydrogen bonds with water molecules, it is often difficult to distinguish the effects caused by intrinsic properties of amino acid ions from those caused by the interactions with water.\(^{42}\) In theory, this question might be addressed by a comparison of gas- and solution-phase reaction products. In practice, such comparison may become difficult considering the technical limitations of solution-phase photooxidation experiments. In addition, a simple comparison of gas- and solution-phase reactions may not be able to provide the information about the effects of individual water molecules on amino acid oxidation.

In fact, the presence of hydrogen-bonded water molecules might be essential and critical for molecule dynamics\(^{43}\) of $^1\text{O}_2$ with some particular amino acids (AAs). The comparison between $^1\text{O}_2$-mediated oxidation of bare protonated/deprotonated histidine and their hydrated species in our recent study exemplifies nicely the importance of water ligands in altering the reactivity of gas-phase species and mimicking the pH-dependence of solution-phase reactions.\(^{44}\) In brief, the gas-phase $^1\text{O}_2$ reaction with His$^+$ leads to no product. This non-reactivity has been elucidated based on our density functional theory (DFT) and Rise-Ramsperger-Kassel-Marcus (RRKM) calculations, as well as dynamic simulations. It is found that both intermediates endoperoxide His-2,5-OO$^+$ and hydroperoxide His-5-OOH$^+$ carried high internal energy and therefore are destined to decay back to reactants. The similar scenario was discovered in the reaction of $[\text{His} - \text{H}]^+ + ^1\text{O}_2$, where "hot" intermediates endoperoxide $[\text{His} - \text{H}]-2,4$-OO$^-$ and
hydroperoxide \([\text{His} - 2\text{H}]\text{-2-OOH}^\cdot\) were unable to survive intact, or evolved to stable products, but decomposed back to reactants.

Surprisingly, stable end oxidation products were produced one histidine was hydrated, which are attributed to 5-hydroperoxide (major) and 2,5-endoperoxide for \(\text{HisH}^\cdot(\text{H}_2\text{O})_{1,2} + {^1}\text{O}_2\), and 2,4-endoperoxide (major) and 2-hydroperoxide for \([\text{His} - \text{H}]\text{(H}_2\text{O})_{1,2} + {^1}\text{O}_2\), respectively. This suggests that the "hot" endoperoxide and hydroperoxide intermediates can remove internal excitation via cluster dissociation and become stabilized with respect to dissociation. Moreover, the reaction efficiencies for dihydrated are higher than their mono-hydrated analogues, due to the fact that the second water is less strongly bound than the first water. Dihydrates therefore have a lower energy dissociation channel to eject a water than monohydrates. These findings reinforce the understanding that gas-phase hydrated AAs involve individual solute-solvent dynamics and their coupling, rather than simply AA dynamics under the influence of some representation of the solvent. In addition, the reactivity of deprotonated hydrates is much higher than that of protonated ones, which reflects the pH dependence observed in solution-phase photooxidation of His.\(^{28b, 45}\) The origin of such reactivity difference can be elucidated by the binding strength of the precursor complexes and the ionization state of the imidazole ring.

The \(^1\text{O}_2\) oxidation of cysteine (Cys) provides another example of the modification of reaction dynamics by the hydrogen-bonded water molecules.\(^{43, 46}\) The oxidation of bare Cys (either protonated or deprotonated) dumped reaction exoergicity into products and caused fragmentation of the Cys moiety. Nevertheless, the addition of water ligands to the system suppressed product dissociation and intrinsically influenced oxidation pathways. Pathways that are normally not feasible in the gas phase become so in Cys-water clusters.

1.3.4 **Final destination: presenting solution-phase reaction kinetics and mechanism**
Experiments on gaseous hydrated clusters not only simplify data interpretations, but provide a microscopic view of more biologically relevant dynamics than gas-phase isolated molecules. On the other hand, gas-phase experiments in explicit "microsolution" still differ from those in an aqueous continuum. For example, the low number density of molecules in the gas phase may prohibit secondary reactions and further conversion of intermediates and primary products. Therefore it is not surprising if the reaction products of gaseous hydrated clusters do not exactly match those in bulk solution. Questions then arise: to what extent the intermediates and products observed in the gas phase resemble or related to the products in aqueous solution, and how to extrapolate gas-phase reaction profiles to solution-phase reactions. More to the point, how reaction dynamics and products evolve from isolated gas-phase species to different hydrated media.

The systematic study to parallel gas- and solution-phase $^{1}O_2$ oxidation on His using ESI-MS provides a successful precedent. The ion-molecule scattering of $\text{HisH}^+/[\text{His - H}]^-$ with $^{1}O_2$ in the gas phase allows us to identify the intrinsic interactions of His and $^{1}O_2$, and $^{1}O_2$ oxidation of hydrated clusters $\text{HisH}^+(\text{H}_2\text{O})_{n=1,2}/[\text{His - H}]^+(\text{H}_2\text{O})_{n=1,2}$ enables us to recognize the effect of individual solvent molecules. However, it is until we obtained the mass spectrometry data in real-time solution-phase $^{1}O_2$ oxidation of His, we could construct an in-depth profile of evolution of various products as a function of reaction time, reveal pH dependence in photooxidation of His, and explore the extent of resemblance and relationship between reactions in the gas-phase and aqueous continuum.

1.3.5 A new on-line ESI-MS approach for $^{1}O_2$ dynamics

Over recent years the application of ESI-MS has spread rapidly in monitoring of solution-phase reactions over the course of the chemical transformations. The fast growth of such
interests relies on several exclusive advantages of ESI-MS compared to other conventional monitoring techniques. 1) The sample collection process is much "cleaner", thanks to the relatively "soft" ionization method, where no/less harsh conditions are applied onto the sample solution being transferred into the mass spectrometer. As a result, weakly bonding species generated in solution during the reaction may survive upon direct transfer into gas phase and subsequent detection by MS. 2) Mass spectrometry is a rapid and sensitive approach that provides chances for probing multiple reaction intermediates/products simultaneously at very low detection limits (nanomolar concentration). It enables the observation of reaction intermediates, which might not survive the normal handling of off-line analysis. 3) Each mass spectrum can represent a snapshot of the reaction status due to the speedy feature of MS measurement. As a result, monitoring of the real time reaction dynamic through a continuous uninterrupted analysis of the reaction mixture can be realized.

A new reaction apparatus, which couples the "clean" \(^1\text{O}_2\) generation, \(^1\text{O}_2\) emission detection and \(^1\text{O}_2\) reactions in solution with ESI-mass spectrometry, has been developed in our lab. Singlet \(\text{O}_2\) was generated using a chemical generator and bubbled into reaction solution, and the reaction solution is continuously transferred into ESI-MS for analysis. Our on-line apparatus minimizes sample transfer time between reaction and mass spectrometry measurements, and enables us to observe real-time kinetics of \(^1\text{O}_2\) with biomolecules in solution. Reaction products may be detected in positive/negative ESI modes and their structures can be characterized using collision-induced dissociation in our tandem mass spectrometer.
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Chapter 2

Experimental Instrumentation and Computational Methodology

2.1 ESI guided-ion beam tandem mass spectrometry

A schematic representation of an ESI, guided-ion beam tandem mass spectrometer used for this experiment is shown in Figure 2.1. The basic concept is similar to guided-ion-beam apparatuses used by other groups.\(^1\) The apparatus can be divided into five sections: (1) ion source; (2) hexapole ion guide; (3) quadrupole mass filter; (4) octopole ion guide and scattering cell; and (5) second quadrupole mass filter and detector. Briefly, ions were generated in an ESI source, thermalized in a hexapole ion guide and mass-selected using a quadrupole mass filter, and then interacted with neutral reactant gas in an octopole ion beam guide. Ionic reactants and products were mass analyzed by a second quadrupole mass filter, and detected.

![Figure 2.1 Schematic diagram of the electrospray ionization guided-ion-beam tandem mass spectrometer](image)

Figure 2.1 Schematic diagram of the electrospray ionization guided-ion-beam tandem mass spectrometer
The sample solution was prepared in suitable solvents with appropriate concentrations ($10^{-3} - 10^{-4}$ M) and sonicated for complete dissolution when necessary. The solution was sprayed into the ambient atmosphere through an electrospray needle at a constant flow rate (0.02 - 0.05 mL/h), using a syringe pump (KdScientific model 100). The electrospray needle was prepared from 35-gauge hypodermic stainless steel tubing (0.13 mm o.d. × 0.06 mm i.d., Small Parts Inc.), and biased at high electric potential relative to ground. The charged droplets formed from the electrospray needle were fed into a heated desolvation capillary through a nozzle (diameter of aperture of the nozzle is 0.37 mm). The assembly of the capillary was biased with electric potential relative to ground and heated to suitable temperatures using heating tape. Charged liquid droplets and solvated ions underwent continuous desolvation as they passed through the heated capillary, converting to gas-phase ions, and then were transported into the next vacuum chamber.

A skimmer is located 2.54 mm from the capillary end, separating the first two vacuum regions. The skimmer was biased relative to ground, and the electrical field between the capillary and skimmer removed remaining solvent molecules attached to ions by collision-induced desolvation, and prevented large solvent clusters from depositing downstream. Ions emerging from the skimmer were passed into a hexapole ion guide, which was evacuated to ~ 15 mTorr. At this pressure range, the mean free path of most ions is around 1 - 2 mm. Therefore, the interaction of ions with background gas (mainly air and solvent molecules) in the hexapole ion guide led to collisional focusing, and thermalization of internal and translational energies of ions. Ions subsequently passed into a quadrupole mass filter. Mass-selected ions were injected into an octopole ion guide, which was operating at 2 - 3 MHz with peak-to-peak of amplitude of 700 V. In addition, DC bias voltage was applied to the ion guide, with the
amplitude varying from -500 to +500 V. The DC bias voltage was used in the retarding potential analysis (RPA)\(^5\) to determine the initial kinetic energy of selected ions, \(i.e.,\) intensities of ions were measured while sweeping the octopole bias. The DC bias voltage also allows control of the kinetic energy (\(E_{lab}\)) of ions in the laboratory frame, thereby setting the collision energy (\(E_{col}\)) between ions and reactant gas molecules in the center-of-mass frame, \(i.e.,\) \(E_{col} = E_{lab} \times \frac{m_{neutral}}{m_{ion} + m_{neutral}}\), where \(m_{neutral}\) and \(m_{ion}\) are masses of neutral and ionic reactants, respectively.

Midway along its length, the octopole is surrounded by a scattering cell. Neutral reactant gas was introduced into the cell through a leak valve, and the cell gas pressure was measured by a capacitance manometer (MKS Baratron 690 head and 670 signal conditioner). Both product ions and the remaining reactant ions were collected by the octopole ion guide, and mass-analyzed by the second quadrupole mass filter.

Both quadrupole mass filters use 9.5 mm diameter rods, operating at 2.1 MHz (Extrel 150 QC) to cover the mass-to-charge ratio range from 1 to 500. Ions were detected by an electron multiplier. The detector signal was amplified by a 100 MHz preamplifier/discriminator, and processed using the standard pulse-counting technique. Ion beam intensity was typically \(4 - 8 \times 10^5\) ion/sec, and constant within 10%. The instrument is interfaced to two National Instruments PCI-6229 multifunction DAQ cards, controlled by LabVIEW\(^6\) programs developed for ion-counting, mass scans, ion kinetic energy calibrations, and cross section measurements. Reaction cross sections were calculated from the ratios of reactant and product ion intensities, calibrated \(^1\)O\(_2\) pressure, the scattering cell gas pressure, and the calibrated length of the scattering cell, using a Beer's Law relationship.

**CID measurements** In order to elucidate the structures of the product ions, collision induced dissociation (CID) experiments were performed with gas Xe (99.995 %, Spectra Gases,
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Stewartsville, NJ, USA). The interested ions were mass-selected by the first quadrupole and passed through the octopole surrounded by the scattering cell containing Xe gas. The Xe gas pressure was maintained at 0.3 mTorr. CID product ion mass-spectra were measured at the center-of-mass $E_{\text{col}} = 0.5$ eV, $1.0$ eV and $1.5$ eV for the product ions. Precursor product ions and their fragment ions were collected by octopole ion guide, mass analyzed by the second quadrupole and counted.

2.2 Generation of "clean" singlet oxygen by a chemical method and its detection

![Schematic diagram of $^1\text{O}_2$ chemical generator and detection system](image)

$^1\text{O}_2$ was generated using the reaction of $\text{H}_2\text{O}_2 + \text{Cl}_2 + 2\text{KOH} \rightarrow \text{O}_2 \left(a^1\Delta_g\right)/\text{O}_2\left(X^3\Sigma_g^+\right) + 2\text{KCl} + 2\text{H}_2\text{O}$. We adopted this technique from Viggiano's group\(^7\) with some modifications. 20 mL of 35 wt.% $\text{H}_2\text{O}_2$ (Acros Organics) was mixed with 10 mL of 8.0 M KOH (> 85%, Fisher Chemical) solution very slowly in a sparger which was immersed in a cold bath maintained at -
19 °C by a Lauda RP890 recirculating chiller. The resulting solution was held at -18 °C to lower the vapor pressure of the solution and prevent the decomposition of H₂O₂, and was degassed before the reaction. A continuous flow of He (T.W. Smith, research grade) was first introduced to the slushy KOH/H₂O₂ mixture at a flow rate of 50 sccm to prevent freezing of the mixture at the exit of the fritted gas aerator inside the sparger. Cl₂ (Sigma-Aldrich, ≥ 99.5%), at a flow rate of 2 - 3 sccm, was then mixed with He in a gas proportioner (Matheson model 7300) and then bubbled through the solution. All of Cl₂ reacted to form either the ground state or excited O₂.⁸ Water vapor in the resulting gas mixture was removed by a second cold trap after the reactor that was kept at -70 °C with a methanol/water/dry ice slush bath. With this generation technique, only ground state O₂, O₂ (a¹Δg), and He would be remained in the downstream gas flow and introduced to the ion-molecule reactions, eliminating O and O₃ contaminants.

The gases from the chemical generator flew through a stainless steel emission cell (2.54 cm i.d. and 10 cm length), where the emission of O₂ (a¹Δg → X³Σg⁻, ν = 0 - 0) at 1270 nm was detected to determine ¹O₂ amount that entered the scattering cell.⁹ The emission cell has a glass window in the front, and a silver-coated concave mirror (ThorLabs CM254-050-P01, f = 50 mm) at the end. The emission cell was continuously pumped by a mechanical pump, and the pumping speed was adjusted using a pressure controller (Cole-Parmer Model R-68027-78 with an intergrated PID) to maintain the emission cell pressure at 15 Torr (measured using a MKS 626B Baratron manometer). This pressure was chosen to reduce the residence time and, hence, the wall quenching of ¹O₂ inside the cold traps and gas tubing, so that maximum emission signal intensity could be achieved. The emission detection system consisted of a 5 nm bandwidth interference filter centered at 1270 nm (Andover, blocked to 1.55 µm), an optical chopper (SRS model SR540), a thermo-electrically cooled InGaAs photodetector (Newport model 71887
detector and 77055 TE-cooler controller), and a digital dual phase lock-in amplifier (SRS model SR830).\textsuperscript{10} \textsuperscript{1}O\textsubscript{2} emission from the emission cell was collected by a plano-convex BK7 lens (ThorLabs LA1805, \(f = 30\) mm), passed through the optical chopper operating at 80 Hz, and the interference filter. The chopped emission was focused by a plano-convex BK7 lens (ThorLabs LA1131, \(f = 50\) mm, AR coated for 1050 - 1620 nm) into the InGaAs detector, and the intensity was measured by the lock-in amplifier.

The detection system measured relative \(\textsuperscript{1}\text{O}\textsubscript{2}\) emission intensity. To determine absolute \(\textsuperscript{1}\text{O}\textsubscript{2}\) concentration, the detector was calibrated using the reaction cross sections of HS\textsuperscript{-} + \(\textsuperscript{1}\text{O}\textsubscript{2}\) \(\rightarrow\) SO\textsuperscript{-} + OH at \(E\textsubscript{col}\) of 0.1, 0.2, and 0.3 eV. HS\textsuperscript{-} was produced by ESI of a methanol/water (5:1 vol. ratio) solution containing a mixture of 0.5 mM H\textsubscript{2}S (\(\geq\) 99.5\%, Ricca Chemical) and 0.1 mM NaOH. HS\textsuperscript{-} anions had a similar initial kinetic energy distribution as that of deprotonated amino acids, and the reaction with \(\textsuperscript{1}\text{O}\textsubscript{2}\) was measured under the identical conditions used for deprotonated amino acids. The rate constant \(k\) for HS\textsuperscript{-} + \(\textsuperscript{1}\text{O}\textsubscript{2}\) \(\rightarrow\) SO\textsuperscript{-} + OH was reported to be \(0.54 \times 10^{-10}\) cm\textsuperscript{3}·molecule\textsuperscript{-1}·s\textsuperscript{-1} by Viggiano et al.\textsuperscript{11} Cross sections \(\sigma\) at different \(E\textsubscript{col}\) can then be calculated using \(\sigma = k/v\textsubscript{rel}\), where the relative ion-molecule velocity \(v\textsubscript{rel} = [2E\textsubscript{col}/ (m\textsubscript{neutral}m\textsubscript{ion}/(m\textsubscript{ion} + m\textsubscript{neutral}))]^{1/2}\).\textsuperscript{12} The calibration indicates that the maximum emission intensity detected corresponds to a 5 - 10\% \(\textsuperscript{1}\text{O}\textsubscript{2}\) yield in the total oxygen flow, which is close to the values reported by Viggiano et al.\textsuperscript{7,13} Since the emission intensity linearly depends on \(\textsuperscript{1}\text{O}\textsubscript{2}\) concentration, the change of \(\textsuperscript{1}\text{O}\textsubscript{2}\) concentration during the experiment could be monitored by measuring the emission. \(\textsuperscript{1}\text{O}\textsubscript{2}\) pressure in the scattering cell is the product of the total gas pressure in the scattering cell, the percent of Cl\textsubscript{2} in the Cl\textsubscript{2}/He flow, and the \(\textsuperscript{1}\text{O}\textsubscript{2}\) concentration in the oxygen product.
To minimize systematic variation in experimental conditions that might be caused by drifting potentials, changes in ion beam intensities, \(^1\text{O}_2\) yield, etc, the emission signal intensity is monitored continuously during the whole experiment, and signal variation is controlled to 20%. Different collision energies were cycled through several times. The RPA measurement of primary ions was performed before and after each experiment to check the initial kinetic energy of the primary ion beam. The entire experiment was repeated several times to check the reproducibility. Based on the reproducibility of previous cross section measurements, we estimated that the relative error of our measurement is < 20%. To check if methionine ions are reactive towards ground state \(\text{O}_2\) and He, we also ran the experiments under the same condition except that \(\text{Cl}_2\) will be replaced by oxygen gas at the same flow rate.

### 2.3 On-line monitoring of aqueous \(^1\text{O}_2\) reactions

A new reaction system (as shown in Figure 2.2) was developed to investigate the mechanisms of \(^1\text{O}_2\)-mediated oxidation of small biomolecules in solution and develop kinetic models. As previously described, \(^1\text{O}_2\) (mixed with \(^3\text{O}_2\) and He) was generated chemically in a sparger (1), of which the water vapor was trapped in a cold trap (2) at -70 °C. The gas-phase emission of \(\text{O}_2\) (\(a^1\Delta_g \rightarrow X^3\Sigma_g^+, \nu = 0-0\)) at 1270 nm was detected in an optical emission cell (3) by a thermoelectrically cooled InGaAs detector (4). To reduce the residence time of \(^1\text{O}_2\) and therefore minimize its wall quenching and self-quenching, the whole generator was continuously evaporated to 25\(\tau\) through a pressure relay (5), before \(^1\text{O}_2\) was bubbled into the aqueous reaction vessel (6). The sample solution was circulated by a peristaltic pump (7). Since a significant amount of water in 6 would evaporate at such reduced pressure, extra water was replenished through an Ismatec Reglo-CPF rotary pump (8) at a precisely controlled flow rate.
Figure 2.3  Schematic diagram of $^1\text{O}_2$ ($\alpha^1\Delta_g$) generation, detection and on-line reaction monitoring system. The ESI-MS is shown for the negative ion mode. For positive ESI-MS, the aqueous sample from loop 11 was directly transferred to stainless steel ESI needle through PEEK tubing.

2.3.1 Determination of steady-state $[^1\text{O}_2]$ dissolved in solution

It is of importance to determine the absolute concentration of dissolved $^1\text{O}_2$ in solution for kinetic analysis. Two $^1\text{O}_2$ chemical traps, 9,10-anthracene diphosphonate dianion (APDA, Sigma-Aldrich) and uric acid (99%, Alfa Aesar), were selected for cross-check. 0.05 mM of the chemical trap was dissolved in water (HPLC grade, submicron filtered, Fisher Scientific). The pH of the APDA solution was adjusted to 10 by adding borax/sodium hydroxide buffer (Fluka),
whereas phosphate buffer (Alfa Aesar) was used for adjusting pH of the uric acid solution to 6.8, respectively. The chemical trap was reacted with constantly replenishing \(^1\)O\(_2\) in 6 and the aqueous reaction solution was continuously circulated through 7 into a quartz flow cell (9) of 1cm optical path length. UV-Vis absorption of the chemical trap was monitored in 9 using an Ocean Optics USB4000 diode array spectrometer (10). During the reaction with \(^1\)O\(_2\), 10 was controlled by software Ocean Optics SpectraSuite so that each spectrum was took and recorded at 30s intervals. The calibration results will be described in Chapter 5.

**2.3.2 On-line reaction monitoring coupled with ESI-MS**

To the best of our knowledge, most of the real-time ESI-MS monitoring approaches were performed for reactions running at a pressure near atmospheric or higher, where reaction solution can be transported to ESI by gravity or positive gas pressure. One requirement of our reaction system, on the other hand, is that the pressure has to be maintained under vacuum (~ 0.5 psi) due to the self-quenching and short lifetime of involved \(^1\)O\(_2\) at atmospheric pressure. This poses a question of how to transport aqueous reaction solution from reduced pressure to open-air ESI. To solve this, a 2-position switching valve (11) coupled with a sampling loop (0.035 mL size) was utilized. The switching valve was programmed by a LabVIEW program. For each measurement, the valve was programed to place in load position for 3 seconds to fill the loop from 6 through 7, and then switched to injection position for transferring the solution out from the loop to electrospray.

In the preliminary study of solution-phase on-line monitoring experiment on the reaction of His + \(^1\)O\(_2\),

\(^1\)O\(_2\), we adopted a sample transfer method from the work reported by Yan and Cooks et al.\(^{15}\) The water from a reservoir swept the sample from the loop to ESI under 4 psi gauge pressure of N\(_2\) gas. To reduce the sample transfer delay time, we also followed their method by
adding an adjustable three-way micro-splitter to the sample line to reduce the amount of sample entering the MS. The sample transfer time was successfully controlled to be fewer than 30s. In the present experiment of Met + ^1O_2 in solution, we have introduced a different approach to transfer the sample to ESI in a more accurate and reproducible manner. Instead of mixing two streams of fluid by the microtee, pulled borosilicate theta tubing was applied as the ESI-emitter. The encounter of sampled aqueous solution and methanol only takes place at the fine tip of the theta tubing (o.d. ~10 µm), and readily submit to ESI after mixing. Consequently, the frequent occurrence of complexities during fluid mixing, i.e., diffusion, turbulence, or segregation, could be avoided, which results in largely enhanced ESI stability as well as effective discharge inhibition. To reduce the lag time of sample transfer and thus further improve the sample collection frequency, syringe pump with programmable flow rates is employed in this study. Accordingly, the residue sample inside the theta tubing after each MS measurement can be withdrawn at controllable speed.

The aqueous sample in the loop was swept with water by a syringe pump (12a) at programmed flow rate and directed into the ESI emitter. To completely remove the residue solution from previous load and subsequently transfer fresh sample to ESI, the sample loop was quickly flushed by water between sample injections, using a programmed syringe pump. The flow rate of the syringe pump 12a was set to 6 mL/h for another 30 s while 11 is at the load position, remained at such after 11 was switched to the injection position, and then set to 0.04 mL/h during MS acquisition. To minimize the length of sample transfer line, at positive ion mode the emitter was assembled by directly gluing the ESI needle (35-gauge stainless steel tubing, 0.005" o.d. × 0.002" i.d., Small Parts, Inc.) into the PEEK tubing (0.0625" o.d. × 0.007" i.d.).
Due to the high surface tension of water, the ESI onset voltage of water solution ($V_{on} = \pm 3.7$ kV) is much higher than that of methanol ($V_{on} = \pm 2.1$ kV) at both ion modes. On the other hand, the onset of electrical discharge at the tip of the stainless steel capillary occurs at lower voltage for negative ion mode ($V_{dis} = -3.6$ kV) than that of positive ($V_{dis} = 4.6$ kV).\textsuperscript{16} As a result, the same ESI emitter described above could not be used at the negative ion mode in our experiment. To avoid the discharge accompanied with conventional metal ESI emitters, Cassou et al. obtained electrospray from water solution by using a borosilicate capillary with tip pulled to the size of i.d. \~{}10 \mu m. The ESI high voltage ($\sim -1.5$ - -1.7 kV) was supplied onto a platinum wire, which was inserted into the sample solution.\textsuperscript{17} However, due to higher flow rate in our experiment, stable ESI could not be achieved with the same setup. Our approach was to utilize a pulled borosilicate theta tubing (13, Sutter Instrument)\textsuperscript{18} as the ESI emitter and mix methanol as a make up solvent with aqueous sample solution at the ESI tip to lower the ESI voltage. The theta capillary was pulled to a tip size of 20 \mu m i.d. \times 40 \mu m o.d. by a micropipette puller (P-2000, Sutter Instrument). Methanol and aqueous sample solution were directed into each channels of the theta capillary through two shreds of PEEK tubing (14a and 14b, 255 \mu m i.d. \times 510 \mu m o.d.), respectively. The ends of 14a/b were inserted into each barrel of the theta capillary, respectively, and fastened by Epoxy gluing. The aqueous sample was swept into one stem of a PEEK microtee (15, Upchurch Scientific), and was led into 13 via 14a. A platinum wire (16, 0.005" o.d., Alfa Aesar) was inserted into the third stem of 15 to supply the electrical connection for ESI of methanol/water mixture. Methanol was driven by a second syringe pump (12b) into 13 at 0.04 mL/h through 14b.

The home-built guided-ion-beam tandem mass spectrometer was used for ESI-MS on-line monitoring of the $^{1}O_{2}$ oxidation of Met, of which the operation, calibration and data analysis
procedures were described above. In brief, an ESI emitter was held at 3.7 and -2.8 kV for producing positively and negatively charged species, respectively. Charged droplets entered the source chamber of the mass spectrometer through a desolvation capillary. The capillary was heated to 130 °C for desolvation. Ions were transported into a hexapole ion guide at a pressure of 26 mTorr, and underwent collisional focusing and cooling to 310 K. Ions subsequently passed into the first quadrupole, which was rendered to an rf-only ion guide during conventional MS measurement, and ions were mass-scanned by the second quadrupole. The typical ion intensity was 300 k/s ~ 500 k/s.

2.4 Computational methods: Electronic structure calculations, RRKM modeling and quasi-classical direct dynamics trajectory simulations

To aid in reaction coordinate interpretation, density function theory (DFT) electronic structure calculations were performed at the B3LYP/6-31+G* levels of theory, using Gaussian 03 for MetH⁺,19 and Gaussian 09 for [Met - H]⁺,20 MetH⁺(H₂O)₁,₂, [Met - H](H₂O)₁,₂ and solution-phase structures.19 Geometries were optimized calculating the force constants at every step. Vibrational frequencies and zero-point energies (ZPE) were scaled by a factor of 0.9613 and 0.9804 for MetH⁺,21 and 0.952 and 0.977 for [Met - H]⁺, MetH⁺(H₂O)₁,₂, [Met - H]⁻(H₂O)₁,₂ and solution-phase structures, respectively. For solution phase calculations, bulk solvation effects were simulated for all the reactants, transition states (TSs), intermediates and products by using the polarized continuum model (PCM) at the same level.20, 22 All the transition states (TSs) found were verified as first-order saddle points by frequency calculations, and the vibrational mode with the imaginary frequency corresponds to the reaction pathway. RRKM rates and density of states were done with the program of Zhu and Hase,23 using its direct count algorithm, and scaled frequencies and energetics from the DFT calculations.
In addition to electronic structure calculations of reactant/product, quasi-classical, direct
dynamics trajectories has been simulated for MetH\(^+\)(H\(_2\)O\(_{0,1}\) + \(^1\)O\(_2\)). We used the chemical
dynamics program VENUS99 of Hase et al.\(^{24}\) to set up the trajectory initial conditions, and the
Hessian based method of Bakken et al.\(^{25}\) implemented in Gaussian 03 to propagate each
trajectory, with Hessian recalculated every five steps.

The initial conditions for the trajectories were chosen to mimic the conditions of our
experiment. Because MetH\(^+\)(H\(_2\)O\(_{0,1}\)) ions were thermalized in the experiment, their initial
vibrational and rotational energies were sampled from Boltzmann distributions at 300 K.
Similarly, \(^1\)O\(_2\) in the experiments was close to room temperature, so 300 K was used for both
rotational and vibrational temperature. The quasi-classical initial vibrational state is simulated
by giving each reactant atom displacement from equilibrium and momentum appropriate to the
initial rovibrational state, with random phases for the different modes. Both MetH\(^+\)(H\(_2\)O\(_{0,1}\))
and \(^1\)O\(_2\) have zero-point energy (ZPE) in all vibrational modes. Trajectories were calculated for
the collision energy of 1.0 eV for MetH\(^+\) + \(^1\)O\(_2\), and 0.1 eV and 0.2 eV for MetH\(^+\)(H\(_2\)O) + \(^1\)O\(_2\).

The integrations were performed with a step size of 0.25 amu\(^{1/2}\)bohr (~0.4 fs), which could
conserve total energy to better than 10\(^{-4}\) Hartree. The SCF = XQC option was used during
trajectory integration, so that a quadratically convergent Hartree-Fock (QC-SCF) method\(^{19,26}\) is
used in case the usual, but much faster, first-order SCF method cannot converge within the
allotted number of cycles.

Because millions of gradients and Hessian evaluations was required, the level of \textit{ab initio}
theory used is necessarily modest. To select a suitable level of theory, we performed relaxed
potential energy surface (PES) scans for approach of \(^1\)O\(_2\) to MetH\(^+\) in several orientations with
various theories and basis sets, and then compared these results to benchmark calculations using
B3LYP/6-311++G** and QCISD/cc-pVDZ levels of theory. On the basis of the overall level of agreement and computational speed, we have chosen the B3LYP/6-21G level of theory for the main set of trajectories.

For MetH+ + 1O2, batches of trajectories (100 each) were calculated for discrete values of the reactant impact parameter \((b = 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 \text{ and } 5.0 \text{ Å})\) rather than randomly sampling the \(b\) distribution. In addition, the random number generator seeds used in setting up initial conditions for each batch of trajectories are identical. Each trajectory batch, therefore, was using the same pseudorandom sequence to sample the reactant parameters (orientations, rotational and vibrational energies, vibrational phases, etc.). As a result, it will be easy to compare trajectories for different impact parameters, because corresponding trajectories from different batches have identical initial conditions, except for the impact parameter being varied. The error from inadequate sampling of reactant parameter space is the same for all batches, and tends to cancel when comparing batches for different impact parameters. For MetH\(^+\)(H\(_2\)O) + 1O\(_2\), we have used the impact parameter \(b = 0.1\) Å for all trajectories, aimed at probing the gross features of reaction dynamics.

All trajectories started with an initial center-of-mass reactant separation of 9.0 Å, and were terminated either when the distance between the final products exceeded 9.0 Å, or after 2000 steps. The error in the energy due to the long-range potential at 9 Å is less than 7 meV. We have to calculate a total of 1200 trajectories, each taking ~200 CPU hours on an Intel quad-core or six-core based cluster. For trajectory visualization we used the program gOpenMol. Detailed analysis of individual trajectories and statistical analysis of the trajectory ensemble were done with programs written for this purpose.
One obvious issue with using the QCT method to probe dynamics is that vibrational energy is not quantized. At the start of the trajectories, vibrational energy is partitioned appropriately to represent the initial internal temperature. Lack of quantization allows unphysical distribution of energy between vibrational modes during and after collisions,\textsuperscript{32, 33} including having trajectories where the final $E_{\text{vib}}'$ is below the zero point level. Because we are working at relatively high collision energies, such obviously unphysical behavior was seldom observed in our trajectories. We note that the main purpose of our trajectory simulations is to probe the gross features of the reaction mechanism, particularly at early times in the collisions; thus errors from classical treatment of the nuclear motions should be minimal.
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Chapter 3

Guided-Ion-Beam and Trajectory Study on the Reactions of Bare Protonated and Deprotonated Methionine with $^{1}$O$_{2}$ in the Gas Phase

3.1 Introduction

$^{1}$O$_{2}$-mediated oxidation dynamics of gaseous protonated and deprotonated methionine is described in this chapter. As the first experiment of my thesis research, the investigation on the reactions of bare Met and $^{1}$O$_{2}$ provides the basis for understanding the intrinsic reactivity and interactions between these reactant species. The ion-molecule reactions being probed in our ESI guided-ion-beam apparatus separate the reaction systems from bulk solution environments. In these reaction systems, the complexities associated with the solution-phase photooxidation of Met (e.g., pH, oxygen concentration, solvent composition, combination of light and sensitizers, competition between radical- and $^{1}$O$_{2}$-mediated reactions) are avoided. As a result, intrinsic reactivity of molecules can be distinguished from solvent effects and counter-ion effects.

In order to study the charge effects on the oxidation dynamics of Met and thus unravel the possible cause of pH dependence observed in solution-phase photooxidation, the reaction of Met in different ionization states (i.e., protonated and deprotonated) are explored. The ESI source can be operated in both positive and negative ionization modes, generating protonated and deprotonated Met (MetH$^{+}$/[Met - H$^{-}$]), respectively. Our guided-ion-beam instrument can be used to detect ions in either charge states, by switching the polarity of DC lenses and mass filter/ion guide pole DC bias, and changing the configuration of the electron multiplier.
By combining gas-phase ion-scattering experiments, statistical modeling and molecule dynamics simulations, this chapter is aimed to unravel oxidation mechanisms and dynamics of gas-phase "bare" Met ions and reveal the influence of the charge states of Met on their reactivity.

3.2 Experimental

The experiments in this chapter were carried out in our guided-ion-beam tandem mass spectrometer, which has been described in detail in chapter 2, along with the operation, calibration and data analysis procedures. Only a brief summary is given here, emphasizing the key operation parameters used (Table 3.1 and 3.2).

Table 3.1  Key operating parameters of the ESI-MS for the reaction of MetH$^+$ + ¹O₂.

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>L-Methionine (≥ 99.5%, Sigma, 5 × 10⁻⁴ M), and hydrochloric acid (Riedel-de Haën, 5 × 10⁻⁴ M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvents</td>
<td>HPLC grade methanol and water (1:1 volume ratio)</td>
</tr>
<tr>
<td>ESI flow rate</td>
<td>0.04 mL/h</td>
</tr>
<tr>
<td>ESI bias voltage</td>
<td>+ 3200 V</td>
</tr>
<tr>
<td>Capillary bias voltage</td>
<td>+ 70 V</td>
</tr>
<tr>
<td>Capillary temperature</td>
<td>170 ºC</td>
</tr>
<tr>
<td>Skimmer bias voltage</td>
<td>+ 11 V</td>
</tr>
<tr>
<td>Pressure in hexapole ion guide</td>
<td>22 mTorr</td>
</tr>
</tbody>
</table>

Table 3.2  Key operating parameters of the ESI-MS for the reaction of [Met - H]$^-$ + ¹O₂.

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>L-Methionine (≥ 99.5%, Sigma, 5 × 10⁻⁴ M), and sodium hydroxide (reagent grade, Fisher 5 × 10⁻⁴ M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvents</td>
<td>HPLC grade methanol and water (4:1 volume ratio)</td>
</tr>
<tr>
<td>ESI flow rate</td>
<td>0.04 mL/h</td>
</tr>
<tr>
<td>ESI bias voltage</td>
<td>- 2500 V</td>
</tr>
<tr>
<td>Capillary bias voltage</td>
<td>- 125 V</td>
</tr>
<tr>
<td>Capillary temperature</td>
<td>150 ºC</td>
</tr>
<tr>
<td>Skimmer bias voltage</td>
<td>- 30 V</td>
</tr>
<tr>
<td>Pressure in hexapole ion guide</td>
<td>24 mTorr</td>
</tr>
</tbody>
</table>
3.3 Results and discussion

3.3.1 Energetic and geometrical feature of gas-phase Met with protonated and deprotonated backbone

Protonated methionine (MetH\(^+\)) may exist in various geometric conformations resulting from the flexibility of its structure. To find the global minimum of its conformational landscape, a grid search method was applied.\(^1\) Since we are only interested in low-energy conformations, we assumed a syn-configuration of the carboxylic acid group and a bifurcated NH\(_3^+\)···O=C intramolecular hydrogen bond in MetH\(^+\).\(^2\) Furthermore, the α-amino nitrogen is assumed to be the preferred protonation site for methionine.\(^3\) We systematically rotated each of the torsion angles along the methionine side chain through 360º at 60º increments to generate all possible conformations. Every conformation so generated was subjected to geometry optimization using Gaussian 03\(^4\) to derive the associate minimum energy conformation. Many of the initial conformations optimized to the same minimum energy structure, and the four lowest energy conformations (within 0.1 eV) were found at the B3LYP/6-31+G\(^*\) level of theory. Their structures and relative energies at 0 K (including ZPE) are summarized in Figure 3.1. These low-lying conformations have strong intramolecular C=O ← NH\(_3^+\) → S charge complexation and hydrogen bond interactions, with a distance of 2.14 - 2.15 Å between the S atom and the closest H atom of the ammonium group and distances of 2.04 - 2.16 and 2.37 - 2.38 Å from the carbonyl O atom to the closest H of NH\(_3^+\) and to the hydroxyl H, respectively. The next group of stable conformations, with weak C=O ← NH\(_3^+\) → S interactions, lie 0.2 eV higher in energy with respect to conformations, A-D, in Figure 3.1. Our low energy conformations, A-D, are consistent with those found by Carl et al.\(^5\) and Bleiholder et al.,\(^3b\) using simulated annealing techniques and by Lioe et al.\(^3c\) using Monte Carlo simulations. According to Carl et al.’s infrared
multiphoton dissociation experiment,\textsuperscript{5} conformations A-D account for all populations of MetH\textsuperscript{+} at 298 K. Our subsequent calculations of the reaction coordinate focused on the lowest energy conformation, \textit{i.e.}, conformation A. It is certainly possible that interconversion between conformations A, B, C, and D might occur during the collision. It seems unlikely, however, that different conformations of amino acids would significantly change their reaction coordinate, and our trajectory simulations of TyrH\textsuperscript{+} + \textsuperscript{1}O\textsubscript{2}\textsuperscript{6} and MetH\textsuperscript{+} + \textsuperscript{1}O\textsubscript{2} confirm this conclusion.

\textbf{MetH\textsuperscript{-}} To locate the global minimum in the conformation landscape of [Met - H]\textsuperscript{-}, a similar grid search method used for MetH\textsuperscript{+} conformation optimization was applied. Each of the torsion angles of the Met backbone was rotated systematically through 360° at 60° increments to generate trial staggered conformations for [Met - H]\textsuperscript{-}. Every conformation so generated was subjected to geometry optimization at B3LYP/6-31+G(d) to derive associated local minimum energy conformation. Many of the initial conformations converged to the same local minimum. These conformations were re-optimized using a larger basis set B3LYP/6-311++G(d,p). A total of 9 stable conformers were found for [Met - H]\textsuperscript{-}. The most significant four are depicted in the top row of Figure 3.1. Each conformer has a number suffix to denote the order of stability, with a percentile population of 54\%, 26\%, 18\% and 2\%, respectively, at 298 K.
Figure 3.1 Low-lying conformations of protonated (MetH⁺) and deprotonated methionine ([Met - H]⁻). The relative energies (eV) of MetH⁺ were calculated (0 K, including ZPE), while those of [Met - H]⁻ (298 K, including ZPE) were calculated at B3LYP/6-311++G(d, p).
3.3.2 Reaction products and cross section of MetH$^+$ + $^1$O$_2$

For the reaction of MetH$^+$ (m/z 150) + $^1$O$_2$, product ions were observed at m/z 148 over the collision energy range of 0.1 - 2.0 eV. At high collision energies, product ions were also observed at m/z 133 and 104. The latter two product ion masses correspond to the elimination of NH$_3$ and of H$_2$O + CO, respectively, from MetH$^+$, with the loss of H$_2$O + CO increasing in importance with increasing collision energy. [MetH$^+$ - NH$_3$] and [MetH$^+$ - (H$_2$O + CO)] are attributed to collision-induced dissociation (CID), and were observed upon collisions with ground-state O$_2$ and Ar, too. In addition, CID product ions at m/z 132 ([MetH$^+$ - H$_2$O]) and 102 ([MetH$^+$ - CH$_3$SH]) showed up at high collision energies, in agreement with previous CID studies. Product ions of m/z 148, on the other hand, were not observed with ground-state O$_2$, Ar, or He. This product channel corresponds to transfer of two hydrogen atoms from protonated methionine to form hydrogen peroxide (H$_2$O$_2$) with $^1$O$_2$, and is referred to as the H2T channel. We also looked at MetH$^+$ + $^1$O$_2$ over the same collision energy range but used microwave discharge generated $^1$O$_2$. Similar product ions were observed. There are various H2T routes leading to three possible product ions, indicated below. The structures for these product ions are given in Figure 3.3, and the corresponding values of $\Delta H_{\text{rxn}}$ are derived from B3LYP/6-31+G* calculations. In principle, the m/z 148 product ions could also be produced through elimination of H$_2$ via CID of MetH$^+$. However, we can discount this probability, except at high $E_{\text{col}}$, since the CID thresholds are calculated to be 1.1 - 2.0 eV for these product ions.

\begin{align*}
^+\text{H}_3\text{NCH(COOH)C}_2\text{H}_4\text{SCH}_3 + ^1\text{O}_2 & \rightarrow \\
\text{H}_2\text{NCH(COOH)C}_2\text{H}_4\text{S}^+ = \text{CH}_2 + \text{H}_2\text{O}_2 & \text{(labeled P1)} \Delta H_{\text{rxn}} = -0.55 \text{ eV} \\
\text{five-membered cyclic-[HNCH(COOH)C}_2\text{H}_4\text{S(CH}_3\text{)]}^+ + \text{H}_2\text{O}_2 & \text{(labeled P2)} \Delta H_{\text{rxn}} = -0.86 \text{ eV}
\end{align*}
Figure 3.2  Cross sections for the production of H$_2$O$_2$ from the reaction of protonated methionine with $^1$O$_2$, as a function of the center-of-mass collision energy.

The reaction cross sections for the H2T channel are given in Figure 3.2, over the center-of-mass $E_{\text{col}}$ range of 0.1 - 2.0 eV. Note that at the time of my first experiment, our emission detector had not been calibrated yet, and the absolute values of cross sections were calculated on the basis of the assumption that the maximum $^1$O$_2$ yield from the chemical $^1$O$_2$ generator is 15%. There may, therefore, exist uncertainty concerning absolute cross sections. However, based on the fact that methionine is highly reactive toward $^1$O$_2$, and the measured reaction cross sections at $E_{\text{col}} = 0.2$ eV (36 Å$^2$) are close to the collision cross section (44 Å$^2$), we believe that the values of reaction cross sections were reasonably estimated. More importantly, this source of uncertainty does not affect the relative cross sections, i.e., the collision energy dependence of cross section, which is our primary interest here. The relative uncertainty is estimated to be ~20%. The reaction is strongly suppressed by collision energy at low $E_{\text{col}}$, becoming negligible at...
$E_{\text{col}} > 1.25$ eV. Note that various CID channels, because of their endoergicities, do not significantly interfere with the H2T channel at low energies. Cross sections level off at the lowest $E_{\text{col}}$. This is most likely artificial, due to the low collection efficiency of slow products at our lowest $E_{\text{col}}$. On the basis of the estimated cross section values, reaction efficiency ($\sigma_{\text{reaction}}/\sigma_{\text{collision}}$) is around 55 - 82% at $E_{\text{col}} = 0.1 - 0.2$ eV, 30% at 0.3 eV, 10% at 0.5 eV, and less than 5% at 1.0 eV and greater. The low efficiency at high $E_{\text{col}}$ may reflect the competition between chemical reaction and physical quenching of $^1$O$_2$, as well as possible bottlenecks in dynamics.

Production of H$_2$O$_2$ from the reaction of methionine with $^1$O$_2$ has been reported in solution-phase photooxidation, and was rationalized by secondary reactions of an intermediate persulfoxide. However, as shown in Figure 1.1, the secondary reactions leading to the formation of H$_2$O$_2$ occur at pH > 6, where Met exist in the zwitterionic or deprotonated form (note pH = 5.74 for Met). Therefore, alternative pathways must be located to make gas-phase reaction results of MetH$^+ + ^1$O$_2$ explicable.

### 3.3.3 Reaction mechanism and pathways of MetH$^+ + ^1$O$_2$

DFT calculation results for the reaction coordinate of MetH$^+ + ^1$O$_2$ are summarized in Figure 3.3, with reactants shown at zero energy. All energetics are calculated at the B3LYP/6-31+G* level of theory. Two weakly bound complexes (complexes RC and PC) and two covalently bound complexes (hydroperoxides H$_{-1}$ and H$_{-2}$) were found. We attempted to locate transition states connecting the complexes to each other and to the products, as shown in Figure 3.3. Complex RC can be characterized as a reactant-like complex, formed by electrostatic interaction and ionic hydrogen bond. This complex has the O$_2$ moiety sandwiched between the ammonium group and the sulfur atom of MetH$^+$, with distances of 2.28 and 1.64 Å for O-O⋯S and O-
O...H$_3$N$^+$, respectively. The binding energy of RC is 0.38 eV with respect to the reactants. Because no rearrangement is required to form a reactant-like complex, it is unlikely that there would be significant activation barriers inhibiting formation of this complex. This conclusion was confirmed by a relaxed potential energy scan running along the dissociation of this complex back to the reactants. Note that, because of a lack of directional covalent or hydrogen bonds between MetH$^+$ and O$_2$, complex RC does not have a well-defined geometry at the energies available in our experiment. This complex is rather floppy, with large amplitude of intermolecular motion. The significance of this complex is that it allows repeated encounters between reactants, increasing the probability of transition to hydroperoxide intermediates. To this extent, complex RC is referred to as “precursor complex” in the following discussion.

![Schematic reaction coordinate for protonated methionine and O$_2$(a$^1\Delta_g$). Energies of complexes, TSs, and products, relative to reactants, are derived from B3LYP/6-31+G* values, including ZPE. The bond distances are shown in angstroms.](image)

Figure 3.3  Schematic reaction coordinate for protonated methionine and O$_2$(a$^1\Delta_g$). Energies of complexes, TSs, and products, relative to reactants, are derived from B3LYP/6-31+G* values, including ZPE. The bond distances are shown in angstroms.
Hydroperoxide H₁ is a covalently bound intermediate MetOOH⁺, which was observed in trajectory simulations (see below). Attempts to optimize intermediate complexes corresponding to persulfoxide (i.e., without transfer of an H atom from -NH₃⁺ to -SOO) converged, instead, to H₁. This indicates an absence of barrier for intramolecular proton transfer from the ammonium group to the H₁ persulfoxide group. According to Mulliken charge population analysis, the -SOOH group carries most of the positive charge (more than 0.9). This demonstrates that an oxidation-induced post-translational modification could not only affect the local proton affinity of amino acid but also changes the site of protonation. It has been reported that oxidation of MetH⁺ to methionine sulfoxide (MetH⁺-O) results in an increase in proton affinity, due to two factors: (1) higher intrinsic proton affinity and stronger ionic hydrogen bonding of the sulfoxide group than the sulfide group and (2) an increase in the ring size formed through charge complexation by the sulfoxide group, which allows more efficient hydrogen bonding compared to the sulfide group.³ᵃ, ¹⁰ As a result, the proton in MetH⁺-O is equally hydrogen bonded to the amino and sulfoxide group. A similar scenario could happen for hydroperoxide H₁, where an eight-membered ring forms a strong hydrogen bond, stabilizing the proton more tightly. Consequently, the proton in H₁ is located much closer to the persulfoxide group (1.04 Å) than to the amino group (1.50 Å). Hydroperoxide H₂ is an analogue of H₁, except that the hydroperoxide group swings away from the amino group. The energy of H₂ (-1.18 eV) is slightly lower than that of H₁ (-1.01 eV), presumably because of the strong S-N bond in H₂ (the S-N bond length is 2.4 Å in H₂ vs 3.87 Å in H₁). We ran trajectory simulations for H₁ for several picoseconds at the HF/3-21G level of theory, with randomly distributed internal energy equivalent to what H₁ would have in reaction. During the trajectory time, H₁ interconverts with H₂ rapidly. Complex PC is product-like, in that two hydrogen atoms have
been transferred to O₂, leaving H₂O₂ electrostatically bonded to dehydromethionine with a
binding energy of 0.34 eV with respect to P₁ products.

Figure 3.3 shows the possible H₂T reaction pathways at low collision energies, as suggested
by the calculations. Hydroperoxides H₁ and H₂ have the right properties to serve as
intermediates for H₂O₂ elimination. The most obvious pathway appears to be reactants →
precursor complex → hydroperoxide H₁ → products. In this pathway, H₁ has to eliminate
H₂O₂ via concerted elimination of -OOH and one of the methyl hydrogen atoms. Elimination of
H₂O₂ is common for allylic hydroperoxides in the presence of a labile hydrogen on a
neighboring atom. However, we were unable to locate a transition state for H₂O₂ elimination
from H₁. We also attempted to locate a product-like geometry following elimination reaction
of H₁; however, all product-like geometries converged back to H₁. These suggest that H₁
may not be a good candidate for direct elimination of H₂O₂, presumably because it has a strong
hydrogen bonding. A slightly more convoluted pathway is reactants → precursor complex →
H₁ → H₂ → TS_H₂ → PC → products; i.e., H₁ interconverts to H₂, followed by
elimination of H₂O₂ from H₂ via an activation barrier (0.41 eV above H₂). No other low
energy pathways were found leading to H₂O₂ elimination from hydroperoxides, although we
certainly cannot exclude the possibility that such pathways exist.

The structures of H₂T product ions could not be determined by the present experiment.
Structures of P₁, P₂ (a five-membered heterocyclic compound), and P₃ (a six-membered
heterocyclic compound) in Figure 3.3 are derived from DFT calculations, and their heats of
formation (ΔH_rxn) are -0.55, -0.86, and -1.52 eV, respectively. Among these products, P₃ is
energetically most favorably. In P₁ and P₂, the positive charge is mostly located on the sulfur
atom, while part of the positive charge is shifted to the ammonium group in P₃. Dissociation of
complex PC directly produces P_1. P_1 may interconvert to P_2 by intramolecular H transfer via TS_P12 followed by ring closure; however, the high energy (1.44 eV above the reactants) and tightness of TS_P12 make this interconversion insignificant at low $E_{\text{col}}$. On the other hand, P_3 can be accessed from P_1 via a much lower energy barrier, TS_P13 (-0.18 below the reactants). Therefore, P_1 and P_2 are expected to be major products at low energies.

To evaluate whether the complexes and reaction pathways identified in DFT calculations can account for the experimental observations at low $E_{\text{col}}$, we used the RRKM program to calculate the lifetime of these complexes as a function of $E_{\text{col}}$. For each complex, all decomposition channels indicated by dashed lines in Figure 3.3 were included. No barrier is expected for decay of precursor complex back to reactants (i.e., no reaction) in excess of the asymptote; thus, an orbiting transition state	extsuperscript{12} was assumed. The rotation quantum number $K$ was treated as active in evaluating the RRKM unimolecular rate constants $k(E, J)$ so that all $(2J + 1) K$-levels are counted,	extsuperscript{13} i.e.,

$$k(E, J) = \frac{d \sum_{K=-J}^{J} G[E - E_0 - E_r(J, K)]}{\hbar \sum_{K=-J}^{J} N[E - E_r(J, K)]}$$

where $d$ is the reaction path degeneracy, $G$ is the transition state sum of states, $N$ is the reactant density of states, $E_0$ is the unimolecular dissociation threshold, and $E_r$ and $E_r^+$ are the rotational energy for the reactant and the transition state, respectively. The orbital angular momentum $L$ was estimated from the collision cross section, i.e., $L = \mu \nu_{\text{rel}} \left( \frac{\sigma_{\text{collision}}}{\pi} \right)^{1/2}$, where $\mu$ and $\nu_{\text{rel}}$ are the reduced mass and relative velocity of collision partners, respectively. Complexes and TSs were described using scaled frequencies, polarizabilities, and momentums of inertia from the DFT calculations. The RRKM lifetime of hydroperoxides is much longer than that of the precursor complex, presumably because the latter is higher in energy. Given the fact that the precursor complex could convert to hydroperoxides, and trajectory simulations showed that
hydroperoxides H_1 and H_2 could interconvert rapidly, these complexes should be regarded as a single intermediate to account for the overall collision time, with the lifetime being roughly the sum of the calculated lifetimes of precursor complexes H_1 and H_2. At collision energies lower than 0.5 eV, the lifetime of this intermediate varies from 10 to 20 ps, longer than the classical rotational period of these complexes which is 4.1-6.8 ps as estimated using the average angular momentum. Note that the intermediate lifetime decreases quickly with increasing $E_{\text{col}}$.

For comparison, we also calculated the direct “fly by” time required for a 5 Å motion at $v_{\text{rel}}$, which is 0.58 ps at $E_{\text{col}} = 0.1$ eV and 0.26 ps at $E_{\text{col}} = 0.5$ eV. Therefore, the complex lifetime is in the right range and has the right $E_{\text{col}}$ dependence. The conclusion is that these complexes, if formed efficiently, could significantly mediate the reaction at least for low energies, and the formation of hydroperoxides is expected to be the rate-limiting step in the mechanism. To estimate the significance of H_1 and H_2 in the reaction pathway, we calculated the relative populations of H_1 and H_2 assuming they are determined by the ratio of the density of states in these two complexes. This assumption is reasonable, since the complexes interconvert rapidly. We used the RRKM program to calculate the complex density of states. It turns out that the ratio of H_2 to H_1 is collision energy dependent, and varies between 40:1 and 30:1 at $E_{\text{col}} < 1.0$ eV. This suggests that the reaction system spends most of the time as H_2 at low energies. We note that the RRKM model only gives the results out of the set of complexes but omits consideration of the complex formation probability. Therefore, whether or not the complex-mediated mechanism is important for this system hinges on the efficient formation of complex during collision. The following trajectory simulations have shed light on this question.

3.3.4 Direct dynamic trajectory simulations of MetH$^+$ + $^1$O$_2$
**Nature of the Trajectories** Trajectories were run at the collision energy of 1.0 eV, using the B3LYP/6-21G level of theory. Roughly 94% of all 900 trajectories belong to direct nonreactive scattering, resulting in conversion of some collision energy into vibrational and rotational energy (i.e., \( T \rightarrow E_{\text{int}} \)). The remaining trajectories either formed electrostatically and hydrogen bonded weak complexes (i.e., precursor complex in Figure 3.3, 1.5%) or formed hydroperoxide complexes (H_1/H_2, 4.5%). These trajectories are assumed to eventually dissociate to H2T products on the basis of the statistical mechanism discussed above, but it is not practical to propagate these trajectories long enough to observe the decay. No direct H2T reaction was observed at \( E_{\text{col}} = 1.0 \) eV. Figures 3.4 and 3.5 demonstrate two trajectories representative of nonreactive and reactive collisions, respectively. Both figures show changes of various distances and potential energy (PE) during the trajectory. The CM distance is the distance between the centers of mass of the collision partners. Figure 3.4 shows a direct, nonreactive scattering, with only one turning point in the relative motion of the reactant centers of mass; i.e., there is no sign of mediation by a complex in this collision. The time scale of the collision is somewhat arbitrary, but three numbers are relevant. The time between trajectory starts and the onset of strong interaction, which depends on reactant orientation, is around 170 fs. The time for reactant approach within 5 Å of the center-of-mass distance is around 130 fs. More importantly, the time period during which MetH^+ and ^1O_2 interact strongly is around 50 fs, as shown by the potential energy spike beginning at \( t \approx 200 \) fs during the trajectory.

Figure 3.5 illustrates a hydroperoxide-forming trajectory, with similar reactant approach time. The \( r(N-H) \) bond length plotted in the figure corresponds to the distance of the abstracted H atom to the ammonium nitrogen, \( r(S-O) \) and \( r(O-H) \) correspond to the S-OO and SOO-H bonds being formed in the hydroperoxide, and \( r(O-O) \) is the bond length of the O_2 moiety. The
oscillations in bond lengths and PE reflect the vibrations of the reactants or products, including ZPE.

![Graph](image)

**Figure 3.4** A representative plot of non-reactive trajectories, showing the variation of potential energy and center-of-mass distance during the trajectory.

We note that the actual hydrogen transfer from -NH$_3$ to the peroxide oxygen, defined as the moment when the $r$(N-H) bond extends by more than twice the amplitude of the vibrational fluctuations, occurs almost simultaneously (within a few fs) with the formation of new S-OO and SOO-H bonds; *i.e.*, formation of hydroperoxide is concerted. This is again in agreement with our finding from electronic structure calculations that persulfoxide of MetH$^+$ would converge to a hydroperoxide spontaneously. No dissociation was observed for the hydroperoxide during the trajectory time (1000 fs). It is interesting to note that, in most hydroperoxide-forming trajectories, H$_1$ is formed in the initial structure, sometimes isomerizing to H$_2$ before trajectory termination.
Dependence on collision orientation  One of the motivations for doing trajectory simulations is to explore the aspects of reaction dynamics that are not experimentally accessible. We investigated the correlation between reaction probability and collision orientations for MetH$^+$ + $^1$O$_2$. To quantify the dependence of reactivity on orientation, we need to define a critical point in each trajectory where orientation can be examined. Here, we take the critical point to be the first time when O$_2$ approaches within 3.0 Å of the S atom of MetH$^+$. For nonreactive trajectories which never reach this critical point, we characterize the collision orientation at the turning point.
of the inter-reactant separation. Strong orientation dependence was found for MetH\(^+\) + \(^1\)O\(_2\). All complex-forming trajectories must have O\(_2\) approach both the S atom and ammonium group simultaneously, forming precursor and hydroperoxide. At b \(\leq 2.0\) Å, only \(~20\%\) of collisions have such orientations at the time when reactants start to collide (and \(40\%\) of these form complexes). The balance of collisions either have O\(_2\) attack the backbone of methionine (33\% of total trajectories) or approach the -CH\(_2\)CH\(_2\)SCH\(_3\) group from the back side of the ammonium group (47\% of total trajectories). The latter two lead to nonreactive scatterings. Trajectory visualization suggests that the critical point is late enough in the trajectory that there is not enough time for significant orientation steering before forming any complex; therefore, the narrow range of optimal orientations could explain the low reaction efficiency observed in experiment at \(E_{\text{col}} = 1.0\) eV. Trajectories show that collisions exclusively between O\(_2\) and the S atom, \(i.e.,\) without attacking the ammonium group at the same time, do not lead to reactive trajectories. This demonstrates that a precursor complex with a hydrogen bond between NH\(_3^+\) and O\(_2\) is essential for the early stage of the reaction.

### 3.3.5 Non-reactivity of \([\text{Met} - \text{H}]^-\) with \(^1\)O\(_2\)

An unexpected result from the experiment is that no oxidation product was observed for dehydrated \([\text{Met} - \text{H}]^-\). To explore the origin of the non-reactivity of \([\text{Met} - \text{H}]^-\), we have mapped out the PES associated with its reaction coordinate. We excluded in the PES the intersystem crossing from \(^1\)O\(_2\) + \([\text{Met} - \text{H}]^-\) to \(^3\)O\(_2\) + \([\text{Met} - \text{H}]^-\), because excited \(^3\)[Met - H]\(^-\) predissociates to NH\(_2\)CH(CH\(_2\)CH\(_2\)S)CO\(_2^-\) + CH\(_3\). As illustrated in Figure 3.6, a weakly bound precursor complex and two covalently bound complexes may form during the collision of \([\text{Met} - \text{H}]^-\) with \(^1\)O\(_2\). We have located the TSs connecting the complexes to each other and to the products. Two oxidation paths can be proposed, both of which involve the common precursor complex.
The precursor complex is formed by electronic interaction and has a binding energy of 0.67 eV with respect to reactants. The precursor is rather floppy, with large amplitude of intermolecular motion. It allows repeated encounters between the reactants, increasing the probability of crossing activation barriers to form covalently bound hydroperoxides [Met - H]OOH\(^{-}\)_1 and [Met - H]OOH\(^{-}\)_2.

The first pathway follows reactants \(\rightarrow\) precursor complex \(\rightarrow\) TS1\(^{-}\) \(\rightarrow\) [Met - H]OOH\(^{-}\)_1 \(\rightarrow\) TS2\(^{-}\) \(\rightarrow\) product-like complex \(\rightarrow\) [Met - 3H]\(^{-}\) + H\(_2\)O\(_2\). After forming the precursor, an H is transferred from -SCH\(_3\) to the O\(_2\) moiety at TS1\(^{-}\), followed by formation of [Met - H]OOH\(^{-}\)_1 (-0.43 eV with respect to the reactants). TS1\(^{-}\) lies 0.37 eV lower than the reactants, suggesting no activation barriers inhibit formation of [Met - H]OOH\(^{-}\)_1. Within [Met - H]OOH\(^{-}\)_1 a second H can be transferred from \(\gamma\)-CH\(_2\) to -OOH via TS2\(^{-}\), resulting in a product-like complex consisting of NH\(_2\)CH(CH\(_2\)=SCH\(_2\))CO\(_2\)^{-} \cdot \cdot H\(_2\)O\(_2\). The product-like complex lies 0.46 eV below the reactants, with the H\(_2\)O\(_2\) molecule hydrogen bonded to the amino group. Dissociation of the product-like complex gives rise to [Met - 3H]\(^{-}\) + H\(_2\)O\(_2\). However, this route can be discounted at low \(E_{col}\) because its reaction enthalpy is 0.18 eV endothermic; and more importantly, this path bears a high barrier of 0.62 eV at TS2\(^{-}\).

Another possible pathway corresponds to reactants \(\rightarrow\) precursor \(\rightarrow\) TS3\(^{-}\) \(\rightarrow\) [Met - H]OOH\(^{-}\)_2 \(\rightarrow\) TS4\(^{-}\) \(\rightarrow\) product-like complex \(\rightarrow\) [Met - 3H]\(^{-}\) + H\(_2\)O\(_2\). In this mechanism, the initial H transfer occurs from \(\gamma\)-CH\(_2\) \((i.e.,\) TS3\(^{-}\), 0.26 eV below the reactants). The resulting hydroperoxide [Met - H]OOH\(^{-}\)_2 is structurally different than [Met - H]OOH\(^{-}\)_1, and is 0.27 eV more stable. [Met - H]OOH\(^{-}\)_2 may transfer second H from -SCH\(_3\) to -OOH via TS4\(^{-}\), followed by H\(_2\)O\(_2\) elimination to yield the [Met - 3H]\(^{-}\) product. We were unable to locate TS4\(^{-}\) using traditional TS searching methods \((e.g.,\) TS, QST2 and QST3 in Gaussian 09). The TS4\(^{-}\) barrier
height was determined using a relaxed potential energy surface scan running along the hydrogen transfer from -SCH$_3$ to the oxygen terminal of -OOH. The PES scan continuously varied the new bond length rOH from 2.73 to 0.99 Å, and optimized all coordinates other than rOH. The PES scan yielded a single barrier associated with hydrogen transfer, with its energy 1.06 eV higher than that of [Met - H]OOH$_{-2}$. The barrier height is similar to that from [Met - H]OOH$_{-1}$ to TS2$^-$. Consequently, this pathway is also disfavored at low $E_{col}$.

One may question that, since there are no activation barriers leading to formation of [Met - H]OOH$_{-1}$ and 2, why neither of these was detected in the experiment. The mechanistic importance of the precursor and hydroperoxide intermediates depends on their lifetimes, so we have used the RRKM theory to calculate the rates for all unimolecular channels leading from these complexes as indicated in the PES. No barrier is expected for decay of the precursor to reactants (i.e. no reaction) in excess of the asymptote, thus an orbiting transition state was assumed. Analysis of RRKM results provides kinetic insights. At $E_{col} \leq 0.2$ eV (where we expect that a complex-mediated mechanism might be important), the dominant decay channel for the precursor complex corresponds to precursor $\rightarrow$ TS1$^- \rightarrow$ [Met - H]OOH$_{-1}$ with a rate constant $k$ of $4 - 6 \times 10^8$ s$^{-1}$, followed by precursor $\rightarrow$ TS3$^- \rightarrow$ [Met - H]OOH$_{-2}$ with $k = 1 - 2 \times 10^7$ s$^{-1}$, while the $k$ for decay back to reactants is less than $1 \times 10^7$ s$^{-1}$. The dominant channel for both [Met - H]OOH$_{-1}$ and [Met - H]OOH$_{-2}$ correspond to "back to the precursor" (because of tight and high TS2$^-$ and TS4$^-$ in the product channels), and their rate constants are $2 \times 10^{11}$ and $1 \times 10^8$ s$^{-1}$, respectively. Since [Met - H]OOH$_{-1}$ has the shortest lifetime compared to others, its mechanistic importance may be doubtful. On the other hand, the lifetimes of the precursor and [Met - H]OOH$_{-2}$ are in the right range to mediate a reaction. Both complexes have a lifetime longer than the direct collision time (~ps, the time required for a 10 Å motion of reactants at $v_{rel}$).
and the classical rotational period of these complexes (which is ~10 ps as estimated using the average angular momentum). Because these two complexes interconvert rapidly compared to their lifetimes, they should be treated as a single complex. Their total lifetime roughly equals the length of time the system is trapped within the potential wells. In that case, the maximum complex lifetime is determined by the rate constant for the precursor decay back to reactants, which is 0.1 µs at low $E_{col}$. The ion time-of-flight within the octopole and the second mass filter is around 10 µs. As a result, these complexes were barely detectable in product mass spectra.

Figure 3.6  Schematic reaction coordinate for deprotonated methionine and $O_2(a^1\Delta_g)$. Energies of complexes, TSs, and products, relative to reactants, are derived from B3LYP/6-31+G* values, including ZPE.

3.4  Conclusions

We have employed guided-ion-beam tandem mass spectrometry to determine the reaction products, cross sections, and collision energy dependence for the reactions of protonated and deprotonated methionine with electronically excited singlet molecular oxygen ($a^1\Delta_g$). DFT calculations were carried out to construct the PES along the reaction coordinates (including
reactants, intermediate complexes, transition states, and various products), analyze thermodynamics and energy barriers, as well as provide insight into the different types of stabilization of protonated species upon oxidation. DFT calculations demonstrate that the -SOO group of MetOOH$^+$ has a higher proton affinity than the amino group, making methionine hydroperoxide the most stable intermediate. Extensive quasi-classical direct dynamics trajectory simulations were performed at collision energy of 1.0 eV. Trajectories demonstrate the importance of a complex-mediated mechanism for H$_2$O$_2$ elimination even at a high $E_{col}$, and reveal a number of interesting dynamics features including sharp orientation dependence for reaction. One of the most surprising results is that no oxidation products were observed in the reactions of deprotonated Met with singlet oxygen, albeit that there are various intermediates formed during the collisions. Similar to the reaction of MetH$^+$, the reactions of [Met - H]$^- + ^1$O$_2$ is mediated by hydroperoxides. However, neither of these nascent hydroperoxides is stable, and destined to fragmentation. The PES of [Met - H]$^- + ^1$O$_2$ shows that all channels leading to stable end-products from these hydroperoxides are blocked by high and tight activation barriers at low $E_{col}$. Consequently, all hydroperoxides decay back to the reactants (within ps), before they reached the ion detector of the mass spectrometer. The present study demonstrates the charge effects on the oxidation mechanism of methionine.
References


Chapter 4

Oxidation of Gas-Phase Hydrated Protonated/Deprotonated Methionine: Bridging the Gas and Solution-Phase Oxidation Chemistry

4.1 Introduction

The previous chapter described an in-depth study on the reactions between “bare” Met (both protonated and deprotonated) and \(^1\text{O}_2\) in the gas phase. For Met\(^+\) + \(^1\text{O}_2\), an H\(_2\)O\(_2\) elimination channel has been identified, and the results are in agreement with photooxidation ability in acidic solution. However, the non-reactivity of [Met - H]\(^-\) + \(^1\text{O}_2\) seems contradictory to photooxidation results in basic solution. To understand the origins of this disagreement, as well as to probe the solvation effects in biological system, we move one step further; from exploring the reaction of gas-phase “bare” Met to investigating those of gas-phase hydrated Met with explicit number of water ligands.

Approaching solution-phase chemistry ESI allows for formation of gas-phase hydrated ions.\(^1\) In our experiments, hydrated Met in both protonated and deprotonated forms (MetH\(^+\)\(\cdot\)\(n\)H\(_2\)O and [Met - H]\(^-\)\(\cdot\)\(n\)H\(_2\)O) could be generated with the number of water ligands \(n = 1 - 2\), using our ESI source. The reaction products and cross sections of MetH\(^+\)\(\cdot\)\(n\)H\(_2\)O and [Met - H]\(^-\)\(\cdot\)\(n\)H\(_2\)O were measured as a function of the hydration number \(n\). As a result, the individual effect of solvent molecules on the reactivity has been revealed. The investigation on \(^1\text{O}_2\) oxidation of hydrated Met with water molecules attached stepwisely acts as a bridge for connecting gas-phase and solution-phase reactions, and can be used to probe the transition of gas-phase hydrated ions to solution chemistry.
Other motivations For hydrated Met, collisions may lead to dissociative excitation transfer, as observed for \(^1\text{O}_2\) and OH\((\text{H}_2\text{O})\) by Viggiano et al.\(^2\). The first step is energy transfer from \(^1\text{O}_2\) to the hydrated cluster, followed by dissociation of the cluster. This process, if confirmed, can be a possible way for protecting hydrated Met against oxidation. Another importance of this experiment lies in the facts that amino acids may exist as \(\text{^+NH}_3\text{-R-COOH} \cdot n\text{H}_2\text{O}\) in atmospheric aerosols and fog waters, and reactions with \(^1\text{O}_2\) contribute to their major loss.\(^3\) However, little is known about their mechanisms and kinetics. The measurements of hydrated species in the laboratory are useful for developing photochemical transformation models of amino acids in the atmosphere.

4.2 Experimental

Table 4.1 Key operating parameters of the ESI-MS for the reaction of Met\(H^+(\text{H}_2\text{O})_{1,2} + \text{^1O}_2\).

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>L-Methionine (≥ 99.5%, Sigma, 5 × 10(^{-4}) M), and hydrochloric acid (Riedel-de Haën, 5 × 10(^{-4}) M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvents</td>
<td>HPLC grade methanol and water (1:1 volume ratio)</td>
</tr>
<tr>
<td>ESI flow rate</td>
<td>0.03 mL/h</td>
</tr>
<tr>
<td>ESI bias voltage</td>
<td>+ 2300 V</td>
</tr>
<tr>
<td>Capillary bias voltage</td>
<td>+ 115 V</td>
</tr>
<tr>
<td>Capillary temperature</td>
<td>130 ~ 140 ºC</td>
</tr>
<tr>
<td>Skimmer bias voltage</td>
<td>21 V</td>
</tr>
<tr>
<td>Pressure in hexapole ion guide</td>
<td>23 mTorr</td>
</tr>
</tbody>
</table>

Table 4.2 Key operating parameters of the ESI-MS for the reaction of [Met - H]\((\text{H}_2\text{O})_{1,2} + \text{^1O}_2\).

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>L-Methionine (≥ 99.5%, Sigma, 5 × 10(^{-4}) M), and sodium hydroxide (reagent grade, Fisher 5 × 10(^{-4}) M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvents</td>
<td>HPLC grade methanol and water (4:1 volume ratio)</td>
</tr>
<tr>
<td>ESI flow rate</td>
<td>0.04 mL/h</td>
</tr>
<tr>
<td>ESI bias voltage</td>
<td>- 1900 ~ - 2000 V</td>
</tr>
<tr>
<td>Capillary bias voltage</td>
<td>- 150 V</td>
</tr>
<tr>
<td>Capillary temperature</td>
<td>130 ~ 140 ºC</td>
</tr>
<tr>
<td>Skimmer bias voltage</td>
<td>- 20 V</td>
</tr>
<tr>
<td>Pressure in hexapole ion guide</td>
<td>19 mTorr</td>
</tr>
</tbody>
</table>
The experiments in this chapter were carried out in our guided-ion-beam tandem mass spectrometer, which has been described in detail in chapter 2, along with the operation, calibration and data analysis procedures. The key operation parameters used for these experiments are listed (in Table 4.1 and 4.2).

4.3 Gas-phase structures of MetH+(H2O)1,2 and [Met - H]-(H2O)1,2

Starting geometries of monohydrated ions were obtained by adding a water to all possible hydration sites in the lowest energy conformations of MetH+ and [Met - H]-, and then optimized at B3LYP/6-311++G(d,p). A similar approach was used to build the hydration shell of other amino acids.6,7 Four low-lying conformers were identified for [Met - H]-(H2O), and are included in Figure 4.1. Hydration energy was calculated using $E_{\text{hydration}} = E(\text{bare ion}) + nE(H_2O) - E(\text{cluster})$, where $E(\text{bare ion})$, $E(H_2O)$ and $E(\text{cluster})$ are the DFT energies of bare ion, water and the hydrate of the same ion conformation, respectively. Although both the carboxylate and amino groups of [Met - H]- can offer binding sites for water, stable conformations prefer to have water hydrogen bonded to the carboxylate. The lowest energy conformer, [Met - H]-(H2O)_1, undergoes bidentate complexation, forming two hydrogen bonds between H2O and COO- via a six-membered cyclic arrangement with a hydration energy of 0.69 eV. This conformer accounts for 95% of the monohydrate at 298 K. Other three conformers are monodentate complexes, all forming a single hydrogen bond between H2O and COO-, with a total population of 5%.

Starting geometries of [Met - H](H2O)_2 were created by combining any two of the hydration sites we have identified in [Met - H](H2O). The first four low-lying conformations are shown in Figure 4.1. The most stable conformer [Met - H](H2O)_2_1 accounts for a 75% population of the dihydrate at 298 K. It has each water form a hydrogen bond with a carboxylate oxygen atom, plus a weaker hydrogen bond between the two waters, with total $E_{\text{hydration}}$ of 1.25 eV. In [Met -
H][(H₂O)₂, 3 and 4, one water simultaneously binds to the two oxygen atoms of -COO⁻ in a manner similar to that in [Met - H][H₂O]₂, the other water either binds to an O atom of -COO⁻ or to the N atom of -NH₂.

Figure 4.1 Conformations of MetH⁺(H₂O)₁,₂ and [Met - H][H₂O]₁,₂ calculated at B3LYP/6-311++G(d,p). Dashed lines indicate hydrogen bonds. Bond distances are in Å. Hydration energies at 0 K (eV, including ZPE) are indicated in parentheses.
Three stable conformations were found for MetH\(^+\)(H\(_2\)O) as shown in Figure 4.1, with a population of 52%, 33% and 15%, respectively. Both the carboxyl and ammonium groups of MetH\(^+\) offer hydration sites. The water oxygen either binds to the -OH site of the carboxyl group with \(E_{\text{hydration}}\) of 0.63 eV, or to one of the three -NH sites of the ammonium group with \(E_{\text{hydration}}\) of 0.61 - 0.64 eV. We tried the conformations of HN···HOH···S where the water bridges the ammonium group and the S atom, but all such starting conformations converged to the most stable conformation MetH\(^+\)(H\(_2\)O)\(_1\).

Structures of MetH\(^+\)(H\(_2\)O)\(_2\) were obtained by adding a second water to each of the stable MetH\(^+\)(H\(_2\)O) structures. The most stable dihydrate, MetH\(^+\)(H\(_2\)O)\(_2\)\(_1\) with a population of 70% at 298 K, has two waters hydrogen bonded to -OH and -NH, respectively. Due to the decreasing effective charge on NH\(_3\)^+ and the increasing repulsion between water ligands, the hydration energy of MetH\(^+\)(H\(_2\)O)\(_2\)\(_1\) (1.22 eV) is 0.05 eV less than the sum of two corresponding monohydrates, i.e., MetH\(^+\)(H\(_2\)O)\(_1\) (0.64 eV) and MetH\(^+\)(H\(_2\)O)\(_2\) (0.63 eV). Our calculated \(E_{\text{hydration}}\) values agrees with Wincel’s experiment\(^{53}\) which reported \(E_{\text{hydration}}\) of 0.68 eV for monohydrated MetH\(^+\) and 1.32 eV for dihydrated MetH\(^+\).

4.4 Reactions of MetH\(^+\)(H\(_2\)O)\(_{1,2}\) + \(^1\)O\(_2\)

4.4.1 Products and cross sections of MetH\(^+\)(H\(_2\)O)\(_{1,2}\) + \(^1\)O\(_2\)

Oxidation products for the reaction of MetH\(^+\)(H\(_2\)O) (m/z 168) + \(^1\)O\(_2\) were observed at m/z 148 and 182. The product channel of m/z 148 can be attributed to the abstraction of two hydrogen atoms from MetH\(^+\)(H\(_2\)O) by \(^1\)O\(_2\) to form hydrogen peroxide, followed by liberation of the water ligand, and is referred to as the H\(_2\)O\(_2\) channel. A similar H\(_2\)O\(_2\) channel was observed for the reaction of MetH\(^+\) + \(^1\)O\(_2\). The product channel of m/z 182 corresponds to formation of MetOOH\(^+\) by elimination of the H\(_2\)O ligand from the reaction intermediate MetOOH\(^+\)(H\(_2\)O).
Cross sections for the two product channels and the total reaction efficiencies are shown in Figure 4.2a over the $E_{col}$ range from 0.1 to 1.0 eV. The cross section is 16 Å$^2$ for $m/z$ 148 and 9 Å$^2$ for $m/z$ 182 at $E_{col} = 0.1$ eV, and drops to 2.1 Å$^2$ for $m/z$ 148 and 6.2 Å$^2$ for $m/z$ 182 at 0.15 eV. The H$_2$O$_2$ channel has much sharper $E_{col}$ dependence than MetOOH$^+$, becoming negligible at $E_{col} \geq 0.2$ eV. Therefore, MetOOH$^+$ dominates the products at all collision energies except the lowest one.

![Figure 4.2](image)

**Figure 4.2**  Product cross sections for the reactions of MetH$^+(H_2O)_{1,2}$ with $^1O_2$, as a function of center-of-mass $E_{col}$. Reaction efficiencies are shown on the right axis.

For MetH$^+(H_2O)_2$ ($m/z$ 186) + $^1O_2$, besides $m/z$ 148 and 182, $^1O_2$-specific products was found at $m/z$ 200, corresponding to formation of monohydrated MetOOH$^+(H_2O)$. Their cross sections and the total reaction efficiency are plotted in Figure 4.2b. Similar to its monohydrated counterpart, the reaction of MetH$^+(H_2O)_2 + ^1O_2$ exhibits exothermic behavior, of which the
cross sections increase with decreasing $E_{\text{col}}$. Note that at the lowest $E_{\text{col}}$ a trace of signal was observed at $m/z$ 218, corresponding to the survival reaction intermediate MetOOH$^+$($H_2O)_2$.

The reactions of Met$^+$($H_2O)_{1,2}$ are significant only at low energies. An interesting finding is that Met$^+$($H_2O)_2$ is more reactive than Met$^+$($H_2O)$. Reaction efficiencies for Met$^+$($H_2O)$ and Met$^+$($H_2O)_2$ are 34% and 54%, respectively, at $E_{\text{col}} = 0.1$ eV. Similar results were reported in the reactions of $^1O_2$ with Cys$^+$($H_2O) vs. Cys$^+$($H_2O)_2$.\textsuperscript{37} Another interesting finding is that, compared to its deprotonated counterparts, the reaction efficiencies of Met$^+$($H_2O)$ and Met$^+$($H_2O)_2$ are 15 and 25 times higher (calculated $E_{\text{col}} = 0.1$ eV), respectively.

In addition to $^1O_2$-specific product ions, we have observed the CID production ions corresponding to elimination of water, ammonia and methyl from the Met$^+$($H_2O)_{1,2}$ reactant ions. Since these are not relevant to $^1O_2$ chemistry, they are not discussed further.

### 4.4.2 Reaction mechanism of Met$^+$($H_2O)_{1,2}$ + $^1O_2$

Among the conformations of Met$^+$($H_2O)$ presented in Figure 4.1, Met$^+$($H_2O)_{1,2}$ are predicted to have a population of 52% and 33%, respectively. Based on the significance of their populations, we have considered both conformers in the reaction mechanism. To differentiate the water binding site in Met$^+$($H_2O)_{1,2}$, we include the termini to which water binds in the formulas, \textit{i.e.}, Met$^+$($H_2O)_{1}$ is referred to as Met$^+$($N$-H$2O)$, and Met$^+$($H_2O)_{2}$ as Met$^+$($C$-H$2O)$, for clarity. For dihydrated Met$^+$, we used Met$^+$($H_2O)_2$ as the reactant structure.

We have reported a reaction mechanism for dehydrated Met$^+$ + $^1O_2$, which involves formation of hydroperoxide intermediate MetOOH$^+$ and its dissociation to a dehydro compound [Met - H]$^+$ and $H_2O_2$. Considering the similarities between the chemistry and products of Met$^+$
and its hydrates, we may reasonably presume that the products of m/z 148, 182 and 200 observed in the reactions of MetH+(H2O)1,2 + O2 correspond to formation of [Met - H]+, and hydroperoxides MetOOH+ and MetOOH+(H2O), respectively. Their structures are shown in Figures 4.3 and 4.4, and the reaction enthalpies calculated at B3LYP/6-31+G(d) are summarized as follows:

\[ \text{MetH}^+(\text{N-H}_2\text{O}) + \text{O}_2 \]

\[ \rightarrow \text{MetOOH}^+/\text{iso-MetOOH}^+ + \text{H}_2\text{O} \quad \Delta H = -0.35/-0.52 \text{ eV} \quad (1a) \]

\[ \rightarrow \text{H}_2\text{NCH(COOH)C}_2\text{H}_4\text{S}^+=\text{CH}_2 \quad (\text{P1}) + \text{H}_2\text{O}_2 + \text{H}_2\text{O} \quad \Delta H = 0.08 \text{ eV} \quad (1b) \]

\[ \rightarrow \text{five-membered cyclic-}[[\text{HNCH(COOH)C}_2\text{H}_4\text{S(CH}_3\text{)}]]^+ \quad (\text{P2}) + \text{H}_2\text{O}_2 + \text{H}_2\text{O} \quad \Delta H = -1.00 \text{ eV} \quad (1c) \]

\[ \rightarrow \text{six-membered cyclic-}[[\text{H}_2\text{NCH(COOH)C}_2\text{H}_4\text{SCH}_2]]^+ \quad (\text{P3}) + \text{H}_2\text{O}_2 + \text{H}_2\text{O} \quad \Delta H = -0.29 \text{ eV} \quad (1d) \]

\[ \text{MetH}^+(\text{C-H}_2\text{O}) + \text{O}_2 \]

\[ \rightarrow \text{MetOOH}^+/\text{iso-MetOOH}^+ + \text{H}_2\text{O} \quad \Delta H = -0.37/-0.54 \text{ eV} \quad (1a') \]

\[ \rightarrow \text{H}_2\text{NCH(COOH)C}_2\text{H}_4\text{S}^+=\text{CH}_2 \quad (\text{P1}) + \text{H}_2\text{O}_2 + \text{H}_2\text{O} \quad \Delta H = 0.06 \text{ eV} \quad (1b') \]

\[ \rightarrow \text{five-membered cyclic-}[[\text{HNCH(COOH)C}_2\text{H}_4\text{S(CH}_3\text{)}]]^+ \quad (\text{P2}) + \text{H}_2\text{O}_2 + \text{H}_2\text{O} \quad \Delta H = -0.31 \text{ eV} \quad (1c') \]

\[ \rightarrow \text{six-membered cyclic-}[[\text{H}_2\text{NCH(COOH)C}_2\text{H}_4\text{SCH}_2]]^+ \quad (\text{P3}) + \text{H}_2\text{O}_2 + \text{H}_2\text{O} \quad \Delta H = -1.02 \text{ eV} \quad (1d') \]

\[ \text{MetH}^+(\text{H}_2\text{O})_2 + \text{O}_2 \rightarrow \text{MetOOH}^+(\text{N-H}_2\text{O}) + \text{H}_2\text{O} \quad \Delta H = -0.20 \text{ eV} \quad (2a) \]

\[ \rightarrow \text{MetOOH}^+(\text{C-H}_2\text{O}) + \text{H}_2\text{O} \quad \Delta H = -0.33 \text{ eV} \quad (2b) \]

\[ \rightarrow \text{iso-MetOOH}^+(\text{N-H}_2\text{O}) + \text{H}_2\text{O} \quad \Delta H = -0.37 \text{ eV} \quad (2c) \]
\[ \rightarrow \text{iso-MetOOH}^+ (C \text{-H}_2 \text{O}) + \text{H}_2 \text{O} \quad \Delta H = -0.48 \text{ eV} \quad (2d) \]
\[ \rightarrow \text{MetOOH}^+ + 2\text{H}_2 \text{O} \quad \Delta H = 0.24 \text{ eV} \quad (2e) \]
\[ \rightarrow \text{iso-MetOOH}^+ + 2\text{H}_2 \text{O} \quad \Delta H = 0.07 \text{ eV} \quad (2f) \]

Figure 4.3  Schematic reaction coordinate for mono-hydrated protonated methionine and \( \text{O}_2 (a^1\Delta_g) \). Energies of complexes, TSs, and products, relative to reactants, are derived from B3LYP/6-31+G* values, including ZPE. The bond distances are shown in angstroms.

The PES associated with the low energy pathways for \( \text{MetH}^+(N\text{-H}_2\text{O}) + ^1\text{O}_2 \) is summarized in Figure 4.3, with the reactants shown near the center at zero energy. \( \text{MetH}^+(C\text{-H}_2\text{O}) \) follows identical reaction pathway as \( \text{MetH}^+(N\text{-H}_2\text{O}) \). \( \text{MetH}^+(N\text{-H}_2\text{O}) \) may form a precursor complex with \(^1\text{O}_2\), with \( \text{O}_2 \) sandwiched between the ammonium group and the -\text{SCH}_3 group. Its binding energy is 0.36 eV. The precursor interconverts to a covalently bound complex \( \text{MetOOH}^+(N\text{-H}_2\text{O})_1 \), of which one proton is transferred from the ammonium group to the -\text{SOO} group,
leading to an eight-membered ring with strong hydrogen bonding. MetOOH(\textit{N-H}_2\textit{O})_2 is an analogue of MetOOH(\textit{N-H}_2\textit{O})_1, except that the hydroperoxide group swings away from the amino group. The energy of MetOOH(\textit{N-H}_2\textit{O})_2 (-0.96 eV) is slightly lower than that of MetOOH(\textit{N-H}_2\textit{O})_1 (-0.79 eV), presumably because of the strong S-N interaction in MetOOH(\textit{N-H}_2\textit{O})_2. Both MetOOH(\textit{N-H}_2\textit{O})_1 and 2 may eliminate the water molecule to form the product ions MetOOH\textsuperscript{+}_1 and 2 (\textit{m/z} 182), respectively.

An energetically feasible pathway to the product ion of \textit{m/z} 148 is depicted in Figure 4.3 as reactants $\rightarrow$ precursor $\rightarrow$ TS1a $\rightarrow$ TS1b $\rightarrow$ PC1 $\rightarrow$ TS3 $\rightarrow$ PC3 $\rightarrow$ P3. Instead of forming hydroperoxides, this pathway eliminates H\textsubscript{2}O\textsubscript{2} at the precursor via two consecutive activation barriers TS1a and TS1b. At TS1a the O\textsubscript{2} abstracts an H atom from -SCH\textsubscript{3}, followed by the second H from the ammonium group at TS1b. TS1a and TS1b are located 0.11 and 0.58 eV below the reactants, respectively. Both H\textsubscript{2}O\textsubscript{2} and H\textsubscript{2}O are hydrogen bonded in the ensuing product-like intermediate PC1 (-0.64 eV). PC1 may cross TS3 to another product-like complex PC3 (-1.92 eV), where a covalent bond is formed between -NH\textsubscript{2} and -CH\textsubscript{2}, yielding a six-membered heterocyclic compound hydrogen bonded with H\textsubscript{2}O\textsubscript{2} and H\textsubscript{2}O. The subsequent dissociation of H\textsubscript{2}O\textsubscript{2} and H\textsubscript{2}O from PC3 yields six-membered heterocyclic product ion P3. TS3 locates 0.27 eV below the reactants.

It is not unreasonable to assume that elimination of H\textsubscript{2}O\textsubscript{2} and H\textsubscript{2}O may take place at PC1 to yield product ion P1 (\textit{i.e.}, H\textsubscript{2}NCH(CO\textsubscript{2}H)CH\textsubscript{2}CH\textsubscript{2}SCH\textsubscript{2}\textsuperscript{+}, \textit{m/z} 148). However, this pathway is not the most favorable at low $E_{col}$ due to the associated 0.08 eV endothermicity. Another possible interconversion of PC1 is to transfer a hydrogen from -NH\textsubscript{2} to -CH\textsubscript{2} (\textit{i.e.} TS2) followed by ring closure to form a five-membered heterocyclic complex PC2 (-1.14 eV), which ultimately dissociates to product ion P2. The overall reaction enthalpy for the path "reactants $\rightarrow$ precursor
\[ \text{TS1a/b} \rightarrow \text{PC1} \rightarrow \text{TS2} \rightarrow \text{PC2} \rightarrow \text{P2 + H}_2\text{O}_2 + \text{H}_2\text{O}^\prime \] is \(-0.29\) eV. However, the associated barrier TS2 (1.17 eV) makes this process impossible in our \(E_{\text{col}}\) range.

The proposed mechanism for hydrated MetH\(^+\) raises a question that if elimination of water molecule could happen at any time of the reaction. A related question is to what extent the existence of H\(_2\)O affects the reaction progress; more specifically, whether it is necessary to remain the water ligand until the last step of the reaction. We could image two scenarios for the PES of Figure 4.3. In the first scenario, the departure of H\(_2\)O takes place at the very early stage, subsequent to formation of the precursor. Since the precursor binding energy is much less than the MetH\(^+\) hydration energy, water elimination of the precursor is endothermic. It follows that both MetOOH\(^+\) and H\(_2\)O\(_2\) channels would shut down at low \(E_{\text{col}}\). For the H\(_2\)O\(_2\) channel, the barriers at TS1a/b would increase by 0.59 eV, rendering the overall barrier 0.48 eV above the reactants. In the second scenario, H\(_2\)O leaves from PC1, and the remaining dehydrate PC1 only has the H\(_2\)O\(_2\) moiety hydrogen bonded, and lies \(-0.26\) eV below the reactants (0.38 eV higher than the original PC1). In this case, all the downstream barriers would be lifted by 0.31-0.49 eV in energy and all subsequent pathways become endothermic. Both scenarios would make the reaction pathways energetically much less favorable, implying the mechanistic importance of H\(_2\)O along the reaction course. This also implies potential protection against Met oxidative damage by water elimination in biological systems.

We have calculated the possibility of eliminating H\(_2\)O\(_2\) only at PC1, labelled as [PC1 - H\(_2\)O\(_2\)] (a hydrated analogue of P1). [PC1 - H\(_2\)O\(_2\)] can either form a five-member ring at [TS2 - H\(_2\)O\(_2\)] or a six-member ring at [TS3 - H\(_2\)O\(_2\)]. But [TS2 - H\(_2\)O\(_2\)] and [TS3 - H\(_2\)O\(_2\)] lie at even higher energies (1.49 and 0.1 eV) than TS2 and TS3, respectively.
Computation results for the reaction of \( \text{MetH}^+(\text{H}_2\text{O})_2 + ^1\text{O}_2 \) are summarized in Figure 4.4. We split the PES into two frames. The portion of PES corresponding to formation of hydroperoxide products are shown in Figure 4.4a, \textit{i.e.}, reactants \( \rightarrow \) precursor \( \rightarrow \) MetOOH\(^+\)(H\(_2\)O)\(_2\)_1 \( \rightarrow \) MetOOH\(^+\)(C-H\(_2\)O)\(_1\)/MetOOH\(^+\)(N-H\(_2\)O)\(_1\) \( (m/z\) 200) \( + \) H\(_2\)O. MetOOH\(^+\)(H\(_2\)O)\(_2\)_1 may interconvert to MetOOH\(^+\)(H\(_2\)O)\(_2\)_2, followed by water elimination to MetOOH\(^+\)(C-H\(_2\)O)\(_2\)/MetOOH\(^+\)(N-H\(_2\)O)\(_2\) \( (m/z\) 200). MetOOH\(^+\)(N-H\(_2\)O)\(_1\) and 2 and MetOOH\(^+\)(C-H\(_2\)O)\(_1\) and 2 may undergo further water dissociation, yielding bare MetOOH\(^+\)_1 and 2 \( (m/z\) 182). All single water elimination channels (eqs. 2a - 2d) are exothermic, and one of the double water elimination channels \( (i.e.\) eq. 2f) is nearly thermal; therefore, both single and double water elimination was observed in the hydroperoxide products.

As calculated in eqs. 2g - 2i, only the product channel of six-membered \textit{cyclic-} \([\text{H}_2\text{NCH(CO}_2\text{H})\text{C}_2\text{H}_4\text{SCH}_2]^+\) (P3) \( + \) H\(_2\)O\(_2\) \( + \) 2H\(_2\)O (eq. 2i) may account for the product ion of \( m/z\) 148 at low \( E_{col} \). This route is depicted in Figure 4.4b, of which the involved intermediate complexes and TSs are dihydrated analogues to those for MetH\(^+\)(H\(_2\)O) \( + \) ^1\text{O}_2. The reaction follows reactants \( \rightarrow \) precursor \( \rightarrow \) TS1a(H\(_2\)O)\(_2\) \( (-0.36\ eV) \rightarrow \) TS1b(H\(_2\)O)\(_2\) \( (-0.52\ eV) \rightarrow \) PC1(H\(_2\)O)\(_2\) \( (-0.55\ eV) \rightarrow \) TS3(H\(_2\)O)\(_2\) \( (-0.15\ eV) \rightarrow \) PC3(H\(_2\)O)\(_2\) \( (-1.91\ eV) \rightarrow \) P3 \( + \) H\(_2\)O\(_2\) \( + \) 2H\(_2\)O. To achieve favorable reaction energetics, both the two water molecules and the H\(_2\)O\(_2\) have to be retained in TS3(H\(_2\)O)\(_2\); the dissociation of N-H\(_2\)O, C-H\(_2\)O or H\(_2\)O\(_2\) would increase this activation barrier by 0.47 (labeled as TS3a in the PES), 0.29 (TS3b) and 0.34 eV (TS3c), respectively. The resulting pathways are illustrated by dark grey lines in Figure 4.4b, all of which are disfavored in our \( E_{col} \) range.
Figure 4.4  Schematic reaction coordinate for dihydrated protonated methionine and O₂(a¹Δg). Energies of complexes, TSs, and products, relative to reactants, are derived from B3LYP/6-31+G* values, including ZPE. The bond distances are shown in angstroms.
4.4.3 Direct dynamics trajectory simulations of MetH\(^+\)(N-H\(_2\)O) + \(^1\)O\(_2\)

A further understanding of the collision dynamics for MetH\(^+\)(N-H\(_2\)O) + \(^1\)O\(_2\) was obtained by examining their trajectories at \(E_{\text{col}} = 0.1\) and 0.2 eV. Two hundred trajectories were completed at each \(E_{\text{col}}\). All trajectories were calculated at \(b = 0.1\) Å using the B3LYP/4-31G(d) level of theory. While some trajectories completed reactions within trajectory simulation times (2-3 ps), a large fraction of the trajectories either remain as complex or belong to non-reactive collisions, i.e., fly-by without forming long-lasting complexes. I exemplify two trajectories in Figures 4.5 and 4.6, both were obtained at \(E_{\text{col}} = 0.1\) eV.
Figure 4.6  A representative plot of $\text{H}_2\text{O}_2$ elimination trajectories: (top) the variation of potential energy and center-of-mass distance between $\text{MetH}^+$ and $\text{O}_2$ moieties during the trajectory, and (bottom) the variation of various bond lengths during the trajectory.

A trajectory producing $\text{MetOOH}^+$ and a separated water molecule with the similar reactants approach time as that for the nonreactive trajectory is depicted in Figure 4.5. In addition to the changes in PE and the CM distances $r(\text{O}_2-\text{MetH}^+)$ and $r(\text{H}_2\text{O}-\text{MetH}^+)$, the lower frame of Figure
4.5 shows the approaching of the O₂ moiety toward the S atom followed by the formation of S-O bond as indicated by the decrease of $r_{SO}$ at ~1060 fs. At ~1120 fs, an H is transferred from -NH₃ to -SOO, as indicated by the changes of $r_{NH}$ and $r_{OH}$. Elimination of water occurs after completion of proton transfer. By the end of the trajectory, water is separated from MetOOH⁺ by 7.3 Å.

Figure 4.6 illustrates a trajectory that eliminates H₂O₂ and H₂O. At 1768 fs, an H is transferred from -SCH₃ to one end of the O₂, followed by transfer of a second H from -NH₃ to the other end of O₂ and subsequent elimination of H₂O₂. The bonds plotted in Figure 4.6 correspond to the breakage of C-H in -SCH₃ and N-H in -NH₃, formation of two H-O in H₂O₂, and the S-O interaction during the trajectory. High frequency oscillations of various bonds reflect the vibrations of the reactants or products.

Trajectory simulations show that all persulfoxide and hydroperoxide complexes formed in trajectories did not decay back to the reactants before the termination of the trajectories (typically 2-3 ps). This indicates that the lifetimes of these complexes are at least no less than the trajectory time. For comparison, the classical rotational period of a complex estimated using the average angular momentum is 2.5 ps at $E_{\text{col}} = 0.1$ eV. The flyby time, taken as the time required for 5 Å motion at the relative speed of reactants, is 0.58 ps at $E_{\text{col}} = 0.1$ eV. The fly-by time gives a measure of how long a direct collision would last at the same $E_{\text{col}}$. Clearly, the complex lifetime is significantly longer than the fly-by time, and comparable to the complex rotational period.
4.5 Reactions of [Met - H][(H₂O)₁₂ + ¹O₂

4.5.1 Products and cross sections of [Met - H][(H₂O)₁₂ + ¹O₂

Figure 4.7 Product cross sections for the reactions of [Met - H][(H₂O)₁₂ with ¹O₂, as a function of center-of-mass $E_{col}$. Reaction efficiencies are shown on the right axis.

For the reaction of [Met - H][(H₂O) ($m/z$ 166) + ¹O₂, product ions include $m/z$ 47, 75, 130, 133, 148 and 180. Product ions of 47, 75, 130, 133 and 148 correspond to elimination of H₂NCH(CH₂CH₂)CO₂, H₂NCHCO₂, CH₃ and H₂O from [Met - H][(H₂O) or its daughter ion [Met - H]⁻, respectively, and their intensities increase at high $E_{col}$. Among these CID channels, water elimination from [Met - H][(H₂O) is the most significant one. These product ions were also observed in the collisions of [Met - H][(H₂O) with ³O₂/He, and therefore could be excluded from ¹O₂ chemistry. Product ions of $m/z$ 180, on the other hand, were only observed in the reaction with ¹O₂, and can be attributed to formation of hydroperoxide [Met - H]OOH⁻. The
cross section of m/z 180 is shown Figure 4.7a, as a function of center-of-mass $E_{col}$. Also shown in the figure is the reaction efficiency (right-hand scale), estimated by $\sigma_{reaction}/\sigma_{collision}$ where $\sigma_{collision}$ is the greater of ion-induced dipole capture cross section and hard-sphere collision cross section.

For the reaction of [Met - H](H$_2$O)$_2$ (m/z 184) + $^1$O$_2$, product ions were observed at m/z 47, 75, 130, 133, 148, 180 and 198. As noted for the reaction of [Met - H](H$_2$O) + $^1$O$_2$, the product ions of m/z 47, 75, 130, 133 and 148 were produced from CID of the reactants ions. Only the products ions of m/z 180 (only significant at $E_{col} \leq 0.2$ eV) and 198 are attributed to $^1$O$_2$-specific products [Met - H]OOH$^-$ and [Met - H]OOH(H$_2$O), respectively. On the basis of the reaction enthalpy calculation (vide infra), formation of dehydrated [Met - H]OOH$^-$ appears to be endothermic for [Met - H](H$_2$O)$_2$ + $^1$O$_2$, and therefore should not be expected in the low energy regime. However, the [Met - H]OOH(H$_2$O) products have low velocities in the lab frame at low $E_{col}$, and are likely to undergo secondary reaction with the neutral gas in the scattering cell, eliminating the remaining water ligand. Consequently, some of [Met - H]OOH(H$_2$O) was converted to [Met - H]OOH$^-$. To correct for the secondary reactions at low $E_{col}$, we lumped the intensities of m/z 180 and 198 together in calculating the cross section as well as the reaction efficiency in Figure 4.7b.

The reactions of [Met - H](H$_2$O)$_{1,2}$ with $^1$O$_2$ are inefficient and strongly inhibited by collision energies. Their reaction efficiencies are only $\sim$2% at the lowest $E_{col}$, becoming negligible at $E_{col} \geq 0.5$ eV. The $E_{col}$ dependence of these reactions suggests that the reactions may be complex-mediated, with complex formation probabilities and/or lifetimes strongly suppressed by $E_{col}$.
4.5.2 Reaction mechanisms of [Met - H'](H2O)1,2 + 1O2

As noted, the oxidation pathway of [Met - H'] moves forward to stable products with the addition of water ligand(s). We first focus on the reaction of [Met - H](H2O) with \(^{1}\text{O}_2\). The PES for [Met - H'](H2O) + \(^{1}\text{O}_2\) is illustrated in Figure 4.8. Similar to that for [Met - H'] + \(^{1}\text{O}_2\), a precursor complex and two hydroperoxides may form between [Met - H](H2O) and \(^{1}\text{O}_2\). Except the additional water ligand, the structures of the complexes and TSs in Figure 4.8 are similar to those for dry [Met - H'] in Figure 3.6. To differentiate the similar species between the dry and hydrated systems, we include a water ligand in the acronyms for hydrated structures, e.g. TS1\(^-(\text{H}_2\text{O})\).

Two possible pathways which may lead to the observed product of \(m/z\) 180 are "reactants → precursor → TS1(\text{H}_2\text{O}) → [\text{Met - H}]\text{OOH}(\text{H}_2\text{O})_1 → [\text{Met - H}]\text{OOH}^-_1 + \text{H}_2\text{O}\", and "reactants → precursor → TS3(\text{H}_2\text{O}) → [\text{Met - H}]\text{OOH}(\text{H}_2\text{O})_2 → [\text{Met - H}]\text{OOH}^-_2 + \text{H}_2\text{O}\". At the early stage of the reaction, [Met - H'](H2O) follows the exactly same routes as [Met - H] does (see Figure 3.6), forming hydrated hydroperoxide [Met - H]\text{OOH}(\text{H}_2\text{O})_1 and/or 2. Both hydrated hydroperoxides may eliminate the water ligand to [Met - H]\text{OOH}^-_1 and 2 (\(m/z\) 180), respectively. Their DFT calculated reaction enthalpies are 0.19 and -0.07 eV, respectively. Therefore, [Met - H]\text{OOH}^-_2 is more energetically favored, and dominates the oxidation product at low \(E_{col}\). While [Met - H]\text{OOH}^-_1 might be expected at high energies, the \(E_{col}\) dependence of product cross section (see Figure 4.7) suggests its contribution is insignificant.
Figure 4.8  Schematic reaction coordinate for mono-hydrated deprotonated methionine and O$_2$ (a$^1\Delta_g$). Energies of complexes, TSs, and products, relative to reactants, are derived from B3LYP/6-31+G* values, including ZPE. The bond distances are shown in Å.

We have considered the possibility of H$_2$O$_2$ elimination from the hydrated hydroperoxides. This could happen either via [Met - H]OOH(H$_2$O)$_1$ $\rightarrow$ TS2′(H$_2$O) $\rightarrow$ product-like complex (where a second H is transferred from $\gamma$-CH$_2$ to -OOH), or via [Met - H]OOH(H$_2$O)$_2$ $\rightarrow$ TS4′ (H$_2$O) $\rightarrow$ product-like complex (where a second H moves from -SCH$_3$ to -OOH). Both routes end up with a product-like complex which lies 0.39 eV below the reactants and consists of hydrogen-bonded [Met - 3H], H$_2$O$_2$ and H$_2$O. This complex may expel H$_2$O$_2$ or water or both, with the overall $\Delta H_{rxn}$ of 0.30, 0.16 and 0.80 eV, respectively. It is therefore less likely to have these pathways contribute to the reaction at low $E_{col}$.

A similar mechanism can be proposed for the reaction of dihydrated [Met - H](H$_2$O)$_2$ + $^1$O$_2$. Reaction enthalpies for possible product channels are listed below, all of which have no
activation barriers above the reactants. Based on the calculated $\Delta H_{\text{rxn}}$ values, the favored products at low $E_{\text{col}}$ belongs to $\text{[Met - H]}\text{OOH}^{-}\text{(H}_2\text{O)}_2$.

$$\text{[Met - H]}\text{(H}_2\text{O)}_2 + ^1\text{O}_2 \rightarrow \text{[Met - H]}\text{-OOH}^{-}\text{(H}_2\text{O)}_1 + \text{H}_2\text{O} \quad \Delta H_{\text{rxn}} = 0.08 \text{ eV} \quad (3a)$$

$$\text{[Met - H]}\text{-OOH}^{-}\text{(H}_2\text{O)}_2 + \text{H}_2\text{O} \quad \Delta H_{\text{rxn}} = -0.16 \text{ eV} \quad (3b)$$

$$\rightarrow \text{[Met - H]}\text{-OOH}^{-}_1 + 2\text{H}_2\text{O} \quad \Delta H_{\text{rxn}} = 0.67 \text{ eV} \quad (3e)$$

$$\text{[Met - H]}\text{-OOH}^{-}_2 + 2\text{H}_2\text{O} \quad \Delta H_{\text{rxn}} = 0.41 \text{ eV} \quad (3f)$$

4.6 Summary of microsolvation effects

4.6.1 Effects on MetH$^+$

For monohydrated MetH$^+\text{(H}_2\text{O)}$, the dominant oxidation channel is elimination of H$_2$O$_2$ and water. It is interesting to figure out if elimination of two molecules could happen at any time of the reaction, and could occur in any orders. A related question is to what extent the existence of H$_2$O and H$_2$O$_2$ will affect the reaction process, and more specifically, is the water ligand necessarily to be retained until the last step through the reaction coordinate, (i.e., the intrinsic effect of microsolvation). Figure 4.9 shows a PES with all the possible elimination of H$_2$O and/or H$_2$O$_2$ along the reaction coordinate of MetH$^+\text{(N-H}_2\text{O)} + ^1\text{O}_2$. 1) The departure of H$_2$O may take place at the very early stage along the reaction coordinate, subsequent to the formation of a precursor complex. As a result, the abstraction of two H atoms from -SCH$_3$ and -NH$_3$ occurs at TS1', however, the energy of TS1' for the remaining dehydrated structure increased by 0.59 eV due to the energy cost of water dissociation, and is 0.48 eV above the reactants, suggesting that H$_2$O has to be retained to render the H transfers feasible at low $E_{\text{col}}$. 2) H$_2$O may possibly leave the reaction after the relocation of the Hs onto the O-O moiety and formation of hydroperoxides, i.e., from PC1 to PC1'. PC1' is an dehydrate analogues to PC1 with H$_2$O$_2$ moiety hydrogen bonded in, which lays -0.26 eV below the reactants and 0.38 eV above PC1. A
candidate reaction pathway leading to products of m/z 148 then becomes: PC1' → TS2'/ TS3' → PC2'/PC3' → P2/P3. Similar to that of PC1' vs PC1, TS2', TS3', PC2' and PC3' are dehydrates of TS2, TS3, PC2 and PC3, respectively. Despite PC2'/PC3' lay -1.14/-1.26 eV below the reactants, the increased activation barriers of TS2' and TS3' (1.66 eV and 0.04 eV, respectively; for comparison, TS2 is 1.17 eV and TS3 is -0.27 eV) make the reaction more difficult; 3) Note that dissociation of H₂O₂ may happen after PC1', which results in P1. As proposed in the reaction of dehydrated MetH⁺ + ¹O₂, it is also possible that P1 interconverts to P2 or P3 via TS2'', TS3'', of which the formation of five-member or six-member ring takes place at TS2'' and TS3'', i.e., PC1' → P1 → TS2''/ TS3'' → P2/P3. Nevertheless, after the system hydrates with water, TS2'' and TS3'' become high barriers with activation energies of 2.01 and 0.41 eV above the reactants, respectively. In summary, none of the reaction pathways/products in Figure 4.9 is energetically favored, suggesting the importance of H₂O in stabilizing the involved transition states along the reaction course.

A complication in thinking about the reactions of hydrated clusters with ¹O₂ is that the water ligands may physically quench ¹O₂ during collisions. In the present experiment, we were not able to directly probe the physical quenching of ¹O₂. The quasi-classical trajectory method we used cannot simulate the physical quenching of ¹O₂, either, because trajectories were confined to the lowest energy singlet potential energy surface. However, trajectory simulations of MetH⁺(H₂O) + ¹O₂ illustrate that at E_{col} = 0.1 and 0.2 eV, only less than 8% and 18% of collisions have ¹O₂ attack the water ligand directly, respectively, and only a fraction of such collisions may actually quench ¹O₂. Therefore, it is less likely that the physical quenching by water would significantly affect the branching of ¹O₂ chemical reactions.
Figure 4.9  Schematic reaction coordinate for mono-hydrated protonated methionine and O$_2$(a$^1 \Delta_g$). Energies of complexes, TSs, and products, relative to reactants, are derived from B3LYP/6-31+G* values, including ZPE. The bond distances are shown in angstroms.

4.6.2 Dynamical role of water ligand

The above scheme may lead to an impression that the water in hydrated Met clusters acts mostly as a spectator in reactions such as rare gas tagging (in rare gas-tagged clusters, rare gas atoms represent very weak perturbations to hosts, and spectra of rare gas-tagged clusters represent structures and dynamics of unperturbed systems)$^{10-12}$ However, this is not the case for oxidation of hydrated protonated Met. A Trajectory showing water-catalyzed proton transfer for MetH$^+(N-H_2O)$ + $^1$O$_2$ was observed as shown in Figure 4.10. Similar to what was observed in the reaction of hydrated Cys with $^1$O$_2$,$^{13}$ this finding demonstrates that the reaction coordinate of MetH$^+$ + $^1$O$_2$ can be altered by the absorbed water. This finding reinforces the understanding that gas-phase hydrated amino acids involve both solute and solvent dynamics and their coupling, rather than simply amino acid dynamics under the influence of some representation of the solvent.$^{14}$
Figure 4.10  A representative plot of a water-assisted proton transfer in the formation of a hydroperoxide from MetH$^+$ (H$_2$O) and $^1$O$_2$.

A trajectory producing MetOOH$^+$ and a separated water molecule is depicted in Figure 4.10. The bonds plotted in Figure 4.10 correspond to the N-H bonds being broken in -NH$_3$, the O-H bonds being broken H$_2$O, and the new O-H and S-O bonds being formed in the product MetOOH$^+$, respectively. High frequency oscillations of various bonds reflect the vibrations of the reactants or products. The bottom frame of Figure 4.10 show a concerted transfer of two protons at ~700 fs; one proton is transferred from -NH$_3$ to water, and simultaneously another from water to -SOO. At the same time, a persulfoxide bond is formed between the O$_2$ moiety and S, leading to MetOOH$^+$ (H$_2$O). Around 1000 fs later, the water swings from -NH$_3$ to -SOOH, forming MetOOH$^+$. This trajectory verifies reaction pathway we have proposed in
Figure 4.3. Elimination of water occurs after completion of proton and hydrogen transfer. By the end of the trajectory, water is separated from MetOOH\(^+\) by 8.5 Å.

4.6.3 Effects on [Met - H]\(^-\)

Hydroperoxide intermediates have been formed during the reactions of \(^1\)O\(_2\) with bare Met in both protonated and deprotonated states as verified by our PES calculations, however, none of these hydroperoxides were stable enough to be observed as end products. In the reaction of MetH\(^+\) + \(^1\)O\(_2\), MetOOH\(^+\) facilitates the intramolecule H transfer from MetH\(^+\) to O\(_2\), an H\(_2\)O\(_2\) elimination channel was observed as a result. Hydroperoxides [Met - H] -OOH\(_-_1\) and/or [Met - H] -OOH\(_-_2\) were formed during the reaction of [Met - H] and \(^1\)O\(_2\) but ultimately decayed back to reactants due to the high internal excitation they carried from the reaction enthalpy.

For comparison, hydroperoxide complexes were detected as final products in the \(^1\)O\(_2\)-mediated oxidation of hydrated Met in both ionization states. For the reaction of \(^1\)O\(_2\) with [Met - H], intermediate hydroperoxides [Met - H] -OOH\(_-_1\) and/or [Met - H] -OOH\(_-_2\) carries high internal excitation, and are destined to decay back to reactants. Hydrates of these hydroperoxides with weakly bound water provide a mechanism by which the energized hydroperoxide product complex can dispose of sufficient internal excitation so that the hydroperoxide moiety does not undergo further dissociation. The water dissociation energy from [Met - H] -OOH(H\(_2\)O) to [Met - H] -OOH\(^-\) is 0.57 eV. This energy amount can be compensated by the reaction enthalpy from the formation of [Met - H] -OOH(H\(_2\)O) in the previous step. Since no activation barrier would be expected for water elimination, this dissociation channel avoids high activation barriers required for H\(_2\)O\(_2\) elimination, and should be more efficient. As a result, a single water molecule could introduce a significant step in the transition of [Met - H] oxidation from the gas phase to the solution, \textit{i.e.}, from non-reactive to reactive.
References

Chapter 5

Mechanistic and Kinetic Study of Solution-phase $^{1}$O$_{2}$ Oxidation of Methionine Using On-line Mass Spectrometric Monitoring Approach

5.1 Introduction

The last project for my thesis research is described in this chapter, which focuses on a detailed study on real-time solution-phase $^{1}$O$_{2}$ oxidation of Met under different pH conditions with corresponding ionization states. Combined with previous chapters, this achieves the ultimate purpose of my thesis research, which is to create a panorama of methionine oxidation dynamics from the gas phase through microsolvation to aqueous solution. To complete the present study, it is necessary to develop a new solution-phase reaction apparatus to overcome several technical difficulties associated with on-line reaction monitoring for $^{1}$O$_{2}$ reactions. The essential for on-line reaction monitoring is to minimize the sample transfer time, so that the reaction progress can be followed in real time. The time lag between sampling and measurement is determined by the amount of sample, the length of the transfer line before ESI, and the sample transfer speed. In the preliminary study of solution-phase reaction of His + $^{1}$O$_{2}$, we adopted a sample transfer method reported by Cooks and coworkers.\(^1\) The water from a reservoir swept the sample from a sample loop to ESI under the pressure of N$_{2}$ gas and an adjustable three-way micro-splitter was added to the sample line to reduce the sample transfer time for ESI-MS.

In this chapter, we have developed a different approach to transfer the sample in a more accurate and reproducible way, improving the ESI-MS quality in both positive and negative ion modes. It satisfies the stability and frequency requirements for MS-sampling, yielding a detailed kinetics of solution-phase methionine oxidation.
The detailed configuration of this newly developed on-line monitoring system has been described in Chapter 2 and the key operating parameters are summarized in next section. By paralleling the gas-phase results we have obtained from ion-molecular reactions of bare and hydrated Met and $^{1}$O$_{2}$ in previous chapters, we were able to construct an in-depth profile of evolution of various products as a function of time, reveal pH dependence in photooxidation of His, and explore the extent of resemblance and relationship between gas-phase reactions and aqueous continuum.

5.2 Experimental

5.2.1 Mass spectrometer parameters

Table 5.1 Key operating parameters of the ESI-MS in monitoring Met oxidation in acidic solution.

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>L-Methionine ($\geq 99.5%$, Sigma, $0.5 \times 10^{-3}$ M), and hydrochloric acid (Riedel-de Haën, $0.5 \times 10^{-3}$ M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvents</td>
<td>HPLC grade water</td>
</tr>
<tr>
<td>ESI bias voltage</td>
<td>$+ 3500$ V</td>
</tr>
<tr>
<td>Capillary bias voltage</td>
<td>$+ 110$ V</td>
</tr>
<tr>
<td>Capillary temperature</td>
<td>$130$ °C</td>
</tr>
<tr>
<td>Skimmer bias voltage</td>
<td>$+ 28$ V</td>
</tr>
<tr>
<td>Pressure in hexapole ion guide</td>
<td>$23$ mTorr</td>
</tr>
</tbody>
</table>

Table 5.2 Key operating parameters of the ESI-MS in monitoring Met oxidation in basic solution.

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>L-Methionine ($\geq 99.5%$, Sigma, $2.5 \times 10^{-3}$ M), and sodium hydroxide (reagent grade, Fisher $2.5 \times 10^{-3}$ M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvents</td>
<td>HPLC grade water</td>
</tr>
<tr>
<td>ESI bias voltage</td>
<td>$- 1500$ ~ $- 1700$ V</td>
</tr>
<tr>
<td>Capillary bias voltage</td>
<td>$- 120$ V</td>
</tr>
<tr>
<td>Capillary temperature</td>
<td>$145$ °C</td>
</tr>
<tr>
<td>Skimmer bias voltage</td>
<td>$- 25$ V</td>
</tr>
<tr>
<td>Pressure in hexapole ion guide</td>
<td>$23$ mTorr</td>
</tr>
</tbody>
</table>
The experiment in this chapter was carried out in our guided-ion-beam tandem mass spectrometer, which has been described in detail in chapter 2, along with the operation, calibration and data analysis procedures. Only a brief description is given here, emphasizing the key operation parameters used for the mass spectrometer (in Table 5.1 and 5.2).

5.2.2 Calibration of solution-phase \(^1\)O\(_2\) concentration vs. its gas-phase emission

It is worth noting that since the lifetime of \(^1\)O\(_2\) (~2 \(\mu\)s) is very short in H\(_2\)O and its travel distance is only ~150 nm,\(^2\) it is less likely that \(^1\)O\(_2\) could diffuse far enough to react with reactant molecules in bulk solution. Consequently, the reaction would mostly take place at the gas-liquid interface of the gas bubble and reaction solution. Considering the low concentration of dissolved \(^1\)O\(_2\) in solution and the continuously replenishing gaseous \(^1\)O\(_2\) and water solvent into the reaction solution, we can reasonably assume a steady-state concentration of \(^1\)O\(_2\)\(^3\) in our system. The prerequisite here is that the amount of gaseous \(^1\)O\(_2\) bubble into the reaction solution is reasonably stable as judged by the \(^1\)O\(_2\) emission intensity (vide infra) and so is the pumping speed.

ADPA is known to react with \(^1\)O\(_2\) chemically to produce endoperoxide via \([4+2]\) cycloaddition and serves as a common probe for \(^1\)O\(_2\) trapping in aqueous solution.\(^4\) Figure 5.1 shows the absorption change of ADPA along the reaction course. By plotting Ln(\(A_t/A_0\)) vs. time a linear relationship was found, where \(A_t\) and \(A_0\) are the ADPA peak absorption at 378 nm at any time and time zero, respectively. This observation indicates that the loss of ADPA follows 1st order reaction kinetics. Therefore an average value of dissolved \(^1\)O\(_2\) concentration in reaction solution can be calculated using the reported chemical reaction rate of APDA (\(k_r = 8.2 \times 10^{-7} \text{ M}^{-1} \text{s}^{-1}\)).\(^4\) The emission of \(^1\)O\(_2\) was continuously monitored during each experiment in the emission cell (shown in Figure 5.1). Figure 5.2 presents a nice linearity between the average
[1O2] in solution calibrated using ADPA and the gas-phase 1O2 emission intensity. The results of Figure 5.2 indicate that a minimum amount of 1O2 needed (50 mV) in order to survive the gas bubbling and react with molecules in solution. It is found that [1O2] increases linearly with the growth of 1O2 emission intensity. Once we established the linear relationship between 1O2 emission intensity and average [1O2] in solution, the [1O2] in subsequent experiment were determined using Figure 5.2.

![UV-Vis absorption spectra of APDA over the course of the reaction with 1O2 recorded at pH 10. Spectra changes reveal that the peak absorption at 378 nm decreased as a function of the reaction time. Mechanistic scheme of the reaction of APDA and 1O2 is plotted. The insert shows the plot of ln(A_t/A_0) as a function of the reaction time, where A_t and A_0 are the absorbance at 378 nm, at any time and time zero, respectively.](image)

Figure 5.1 UV-Vis absorption spectra of APDA over the course of the reaction with 1O2 recorded at pH 10. Spectra changes reveal that the peak absorption at 378 nm decreased as a function of the reaction time. Mechanistic scheme of the reaction of APDA and 1O2 is plotted. The insert shows the plot of ln(A_t/A_0) as a function of the reaction time, where A_t and A_0 are the absorbance at 378 nm, at any time and time zero, respectively.

We have also used uric acid as chemical trap to test our solution 1O2 reaction setup, confirmed that the 1O2-induced uric acid oxidation follows first-order kinetics. This again supports pseudo steady state concentration of 1O2 in our solution reaction systems. However, the results in previous kinetic of oxidation of uric acid was complicated by the mixed photosensitization mechanisms. Mantana et al.\textsuperscript{5} reported that photooxidation of uric acid is the mixed process of 1O2 and O2\textsuperscript{−}-mediated oxidation, though the former is more efficient. Rabello
et al. claimed that the reaction between $^1$O$_2$ and uric acid is system-dependent and often consisted of 1st and 2nd order kinetics. Therefore, the rate constant reported for $^1$O$_2$-induced oxidation of uric acid does not represent the exclusive chemical reaction, which results in relatively higher singlet oxidation concentration compared to its real value.

5.3 Results of Met oxidation in basic solution

5.3.1 Reaction products

The basic reaction solution was buffered at pH 10.4 by adding equal molarity of NaOH (0.5 mM) to the Met aqueous solution. Considering the pI and pK$_a$ values of Met (pI of 5.75, and amino pK$_a$ of 9.28), the deprotonated [Met - H] is the major species in this basic solution. Two major $^1$O$_2$-specific product ions were found at m/z 164 and 313, corresponding to deprotonate [Met - H]$^-$-O and dimeric product [Met - H]$^-$-O-Met, respectively. As shown in Figure 5.3, the intensities of these two products increase along the reaction time. It might be possible that the ions of m/z 164 may have contribution from doubly charged dimer [Met - H]$^-$-OO-[Met - H]$^-$. 

Figure 5.2 The linear correlation between the emission intensity of airborne $^1$O$_2$ and the calibrated $^1$O$_2$ concentration in solution using ADPA.
However, this possibility has been excluded based on our CID result of m/z 164, which produced no fragment ions in m/z range higher than 164. On the other hand, CID of ion m/z 313 resulted in two fragments with m/z 164 and 148, respectively, which confirms that this species arises from addition of a neutral Met molecule to deprotonated [Met - H]-O species.

![Mass spectra for Met oxidation in pH 10.4 solution as a function of reaction time.](image)

Figure 5.3 Mass spectra for Met oxidation in pH 10.4 solution as a function of reaction time.

It is noticed that a trace of ions with m/z 180 was also detected. Persulfoxide R₂SOO is usually reported as intermediates formed during ¹⁰₂ oxidation of dialkyl sulfides R₂S, which readily react with a second molecule to yield final products R₂SO. The species we observed at m/z 180 could possibly be attributed to surviving persulfoxide intermediate [Met - H]⁻-OO. One compilation associates with detection of m/z 180 is that methanol was used as make-up solvent to mix with aqueous reaction solution in negative ESI-MS. As a result, [Met - H]⁻/MeOH adduct are expected in ESI. This species has the same m/z as that of persulfoxide intermediate [Met - H]⁻-OO. A question arises regarding the origin of m/z 180, does it consist of the mixture of [Met - H]⁻/MeOH adduct and minor [Met - H]⁻-OO, or just [Met - H]⁻/MeOH adduct alone?
Moreover, even if [Met - H]⁻-OO does exist in this peak, how likely we can extract it from the background [Met - H]⁻/MeOH adduct?

To identify the composition of the species at m/z 180, a control experiment was performed using ethanol as the make-up solvent, i.e., the reaction solution was introduced in the theta tubing to mix with EtOH. The peak of m/z 180 was still visible in the product MS, which indicates the existence of intermediate species [Met - H]⁻-OO and its survival in basic solution but with much lower ion intensity. As shown in Figure 5.3, nevertheless, no obvious time-dependence was observed.

5.3.2 Reaction kinetics


\[ [\text{Met} - \text{H}]^- + ^1\text{O}_2 \rightleftharpoons [\text{Met} - \text{H}]^- - \text{OO} \quad (1) \]

\[ [\text{Met} - \text{H}]^- - \text{OO} + [\text{Met} - \text{H}]^- \xrightarrow{k_2^\text{-}} 2[\text{Met} - \text{H}]^- - \text{O} \quad (2) \]

The rate constants are \( k_1^- \) and \( k_1^+ \) for the forward and reverse reactions of the equilibrium in reaction (1). Since the concentration of [Met - H]⁻-OO rose from zero after the initial induction period and had no obvious change during the major part of the reaction; we have assumed the quasi-steady-state approximation (QSSA)\(^8\) for this species, to simplify the calculation of the reaction rate. Consequently, we have

\[
\frac{\text{d}[[\text{Met} - \text{H}]^- - \text{OO}]}{\text{dt}} = k_1^\text{-}[[\text{Met} - \text{H}]^-][^1\text{O}_2] - k_1^\text{+}[[\text{Met} - \text{H}]^- - \text{OO}]
\]

\[ -k_2^\text{-}[[\text{Met} - \text{H}]^- - \text{OO}][[\text{Met} - \text{H}]^-] = 0 \quad (3) \]

Therefore,
\[
\left[\text{[Met - H]}^{-0}\right] = \frac{k_1\left[\text{[Met - H]}\right][^1\text{O}_2]}{k_2\left[\text{[Met - H]}\right] + k_1'}
\]  
(4)

Based on the PES to be discussed below, we can conclude that for this sequence of two consecutive elementary reactions, reaction (2) acts as the rate-limiting step, and the oxidation scheme involves a pre-equilibrium, in which the peroxide intermediate [Met - H]^{-0} is in equilibrium with the reactants, (i.e., \(k_1' \gg k_2\left[\text{[Met - H]}\right]\)), the rate of decay of [Met - H]^{-0} back into reactants is much faster than the rate at which it forms [Met - H]^{-0}. Then we can neglect \(k_2\left[\text{[Met - H]}\right]\) in the denominator and obtain

\[
\left[\text{[Met - H]}^{-0}\right] = \frac{k_1\left[\text{[Met - H]}\right][^1\text{O}_2]}{k_1'} = K'\left[\text{[Met - H]}\right][^1\text{O}_2]
\]  
(5)

where \(K' = \frac{k_1'}{k_1}\). The net rate for formation of [Met - H]^{-0} is

\[
\frac{1}{2} \frac{d\left[\text{[Met - H]}^{-0}\right]}{dt} = k_2\left[\text{[Met - H]}^{-0}\right]\left[\text{[Met - H]}\right] = K'k_2\left[\text{[Met - H]}\right]^2[^1\text{O}_2]
\]

\[
= k'\left[\text{[Met - H]}\right]^2[^1\text{O}_2]
\]  
(6)

where the effective rate constant \(k' = K'k_2\)

At anytime the total concentration of methionine was conserved, i.e., \([\text{[Met - H]}] + [\text{[Met - H]}^{-0}] = [\text{[Met - H]}]_0\), since the concentration of [Met - H]^{-0} and [Met - H]^{-0}-Met are minimal.

\[
\frac{d\left[\text{[Met - H]}^{-0}\right]}{dt} = 2k'\left([\text{[Met - H]}]_0\right)\left[\text{[Met - H]}^{-0}\right]^2[^1\text{O}_2]
\]  
(7)
Since \(^{1}O_2\) was held constant in the experiment, eq. 7 becomes a pseudo-second-order reaction.

Rearrange the eq. 7 to

\[
\frac{d\left[[\text{Met} - \text{H}^\cdot] - \text{O}\right]}{\left[[\text{Met} - \text{H}^\cdot] - \text{O}\right]_0 \left[[\text{Met} - \text{H}^\cdot] - \text{O}\right]^2} = 2k \left[{^{1}O_2}\right] dt
\]  

(8)

The integrated rate law from \(t = 0\) to reaction time \(t\) is

\[
\frac{1}{[[\text{Met} - \text{H}^\cdot]]_0} \frac{1}{[[\text{Met} - \text{H}^\cdot] - \text{O}]} = 2k \left[{^{1}O_2}\right] dt
\]

(9)

which can be rearranged to

\[
\frac{[[\text{Met} - \text{H}^\cdot]]_0}{[[\text{Met} - \text{H}^\cdot]]} = 2k \left[{^{1}O_2}\right][[[\text{Met} - \text{H}^\cdot]]_0 t + 1
\]

(10)

In our experiment, the initial concentration \([\text{Met} - \text{H}^\cdot]_0\) was set at 2.5 mM, and ESI-MS measurements could reasonably represent the relative ratio of reactant and product ions in the reaction solution. Therefore, concentration of the product can be obtained from the relative ratio of product (P) and reactant (R) as

\[
[[\text{Met} - \text{H}^\cdot]] = [[\text{Met} - \text{H}^\cdot]]_0 \times \frac{R}{R+P}
\]

(11)

the reaction rate constant \(k_1\) can be then determined by

\[
\frac{P}{R} = 2k \left[{^{1}O_2}\right][[[\text{Met} - \text{H}^\cdot]]_0 t
\]

(12)

Note that the ESI-MS of the Met sample had some methionine sulfoxide impurities, and this portion was corrected for in data analysis. The plot of \(P/R\) vs \(t\) fits in to a linear relationship, yielding \(k\) value of \(2 \times 10^9 \text{ M}^{-2}\text{s}^{-1}\).

Kinetics for the formation of \([\text{Met} - \text{H}^\cdot] - \text{O-Met} (m/z 313)\) is due to the secondary reaction of prime product \([\text{Met} - \text{H}^\cdot] - \text{O}\) and another Met.

\[
[\text{Met} - \text{H}^\cdot] - \text{O} + \text{Met} \rightarrow [\text{Met} - \text{H}^\cdot] - \text{O-Met}
\]

(13)
As shown in Figure 5.4, the relative abundance of [Met - H]⁻-O-Met is only 0.4 % after 1-hr reaction, which is much less significant compared to that of [Met - H]⁻-O (ultimate abundance is 3 %). Consequently the concentration depletion of [Met - H]⁻-O due to its interconversion to [Met - H]⁻-O-Met can be safely ignored in calculating $k_3^-$ value in reaction (13).

As a result, we can obtain the value of $k_3^-$ using derivative of [Met - H]⁻-O-Met as a function of time.

$$
\frac{d[\text{[Met-H]⁻-O-Met}]}{dt} = k_3^-\text{[Met-H]⁻-O}[\text{Met}]
$$  

(14)

Note that the absolute concentrations of [Met - H]⁻-O and [Met - H]⁻-O-Met were obtained by their ion abundance as described above, while 2.5 mM is used as total [Met]. The average value of $k_3^-$ is determined to be 1.4 M⁻¹·s⁻¹. Because the abundance of neutral Met is only 7% of total Met species at pH 10.4, it is possible that [Met - H]⁻-O reacts with another deprotonated [Met - H], and the resulting actually doubly charged [Met - H]⁻-O-[Met - H] gained a proton in ESI-
MS measurement. In that case, the reaction rate constant $k_3$ between [Met - H]-O and [Met - H]$^-$ would be $1.09 \times 10^{-1}$ M$^{-1}$·s$^{-1}$.

### 5.3.3 Reaction mechanisms

![Figure 5.5](image)

Figure 5.5  (Top) Relaxed potential energy surface scan from the reactants of [Met - H]$^-$ and $^1$O$_2$ to the persulfoxide [Met - H]-OO intermediate.  (Bottom ) 2D relaxed potential energy surface scan from [Met - H]-OO + [Met - H]$^-$ $\rightarrow$ 2 [Met - H]-O. The calculation was carried out at B3LYP/6-31+G(d) level of theory, including the solvation effects using PCM model.
To elucidate the mechanism accounting for the reaction in basic solution, we have mapped out the reaction coordinate from [Met - H]$^+$ + $^{1}$O$_2$ to [Met - H]$^-$-OO using relaxed potential energy surfaces scan. The solvent effects were included in the calculation using the PCM model. As illustrated in Figure 5.5, the potential energy was continuously monitored while approaching O$_2$ moiety to the sulfur atom of the Met. The initial distance of O-S was set at 7.0 Å and eventually decreased to 1.65 Å (when persulfoxide intermediate [Met - H]$^-$-OO formed) at a step size of 0.05 Å. [Met - H]$^-$-OO lays 0.68 eV below the reactants. No activation barriers were observed above the reactants for forming [Met - H]$^-$-OO.

An activation barrier was located when the formed [Met - H]$^-$-OO reacts with another [Met - H]$^-$ as shown in the bottom of Figure 5.5. At this barrier [Met-H]$^-$-OO breaks its O-O bond and transfers an oxygen atom to the sulfur atom of the approaching [Met - H]$^-$ and produces two [Met - H]$^-$-O as a result. The activation barrier lays 0.40 eV above the reactants of [Met-H]$^-$-OO + [Met - H]$^-$ . This route demonstrates the reaction in eq. (1) and (2), and the corresponding reaction enthalpy is -3.05 eV. Another possible conformation of product with m/z 180 can be attributed to sulfone [Met - H]$^-$-OSO species. The calculated enthalpy is -3.81 eV with respect to reactants, which is even more stable than that of [Met - H]$^-$-O. This species, if formed during reaction, expected to be observed with considerable intensity, which is not in agreement with our experiment results. The reason for this inconsistence could be that a very tight transition state is involved while evolution from persulfoxide [Met - H]$^-$-OO to sulfone species [Met - H]$^-$-OSO. Therefore, the possibility of formation of [Met - H]$^-$-OSO species can be ruled out.

Our initial guess for the geometry of [Met - H]$^-$-O-Met dimer was that either the sulfur atom of Met binds to the sulfur of [Met - H]$^-$-O via a S-S bond, or the two moieties are connected via a bridging S-O-S bond. A grid search method was used to find global minima in the confirmation
landscapes. Each of the torsion angles of the Met backbone was rotated systematically through 360° at 30° increment to generate trial staggered confirmations. To our surprise, instead of forming covalently-bonded complex, all of the trial confirmations for \([\text{Met} - \text{H}]^{-}\text{O-Met}\) converged to hydrogen-bonded structures between Met and \([\text{Met} - \text{H}]^{-}\text{O}\). The most stable confirmation lays -0.56 eV with respect to the reactants and is shown in Figure 5.6.

![Figure 5.6](image.jpg)

Figure 5.6 Reaction coordinate for the reaction of \([\text{Met} - \text{H}]^{-}\text{O} + \text{Met}\) to form dimeric \([\text{Met} - \text{H}]^{-}\text{O}\).

### 5.4 Results of Met oxidation in acidic solution

#### 5.4.1 Reaction products and kinetics

The acidic reaction solution was prepared by adding equal molarity (0.5 mM) of hydrochloric acid into Met aqueous solution, and the pH was measured to be 3.2. Considering the \(p\text{I}\) and \(p\text{K}_a\) values of Met (\(p\text{I}\) of 5.75 and carboxyl \(p\text{K}_a\) of 2.13), the neutral Met is the major species in our acidic solution that participates in the reaction. Only one \(^1\text{O}_2\)-specific product was detected at \(m/z\) 166 in the oxidation of methionine, of which the intensity increases with the reaction time. This product can be attributed to either the protonated methionine sulfoxide product \((\text{MetH}^+ - \text{O})\)
or a protonated dimeric species MetH⁺-OO-MetH⁺. We have performed CID of \( m/z \) 166 with Xe at \( E_{\text{col}} = 1.0 \) eV, and expected that MetH⁺-OO-MetH⁺ if produced in the oxidation, could lose a proton in CID. However, CID of \( m/z \) 166 led to none products in \( m/z \) range higher than 166. Therefore, the structure of a doubly protonated MetH⁺-OO-MetH⁺ can be ruled out for \( m/z \) 166.

Figure 5.7 Mass spectra for Met oxidation in pH 3.2 solution as a function of reaction time.

The formation of Met-O in acidic pH condition has been reported in the study by Sysak and Foote et al,\(^{10}\) and they concluded that the reaction follows 2:1 stoichiometric ratio of Met : \( ^1\text{O}_2 \). The reaction involves the initial formation of intermediate persulfoxide (Met-OO) complex while the consumption of Met and \( ^1\text{O}_2 \) has 1:1 stoichiometry as shown by eq. (14) and (15) listed below,

\[
\text{Met} + ^1\text{O}_2 \rightleftharpoons \text{Met-OO} \tag{15}
\]

\[
\text{Met-OO} + \text{Met} \rightarrow 2\text{Met-O} \tag{16}
\]
Due to the high reactivity of Met-OO,\textsuperscript{51} once formed, it readily reacts with another molecule of Met to yield final product Met-O. The overall reaction rate is determined by the first step. This agrees with the absence of Met-OO in our product MS, and therefore allows us to perform quasi-steady-state approximation on Met-OO for determining kinetics.

Similar as the kinetics analysis for its anion counterpart, the $k$ value for neutra\(l\) Met can finally be determined using eq. (17), where $R$ and $P$ are the fractions of reactant and product in total measured ion intensity, respectively.

\[
\frac{P}{R} = 2k[^{1}O_{2}][Met]_{0}t \tag{17}
\]

where $k = \frac{k_{1}k_{2}}{k_{1}'}$, $k_{1}$ and $k_{1}'$ represent the rate constants for the forward and reverse reactions of the equilibrium in reaction (15). The value of $k$ is measured to be $6 \times 10^{9}$ M$^{-2}$ s$^{-1}$, on the basis of the reproducibility of the kinetics measurements taken over a 3-week period.

\textbf{5.4.2 Reaction mechanisms}

Similar as in basic solution, the potential energy surface of Met + $^{1}$O$_{2}$ was mapped out using relax potential energy surface scan as illustrated in Figure 5.8. Persulfoxide Met-OO was located -1.0 eV lower with respect to the reactants. Met-OO is covalently bound persulfoxide complex, and has O-O sandwiched between the ammonium group and the S atom of the Met moiety, with one H of the ammonium group shuttling between the -NH$_{2}$ and O-O. When another molecule of Met approaches the persulfoxide, an O atom is transferred from Met-OO to Met. Two molecules of Met-O were therefore formed as a result of O-O bond rupture and S-O bond formation. ($\Delta H = -2.21$ eV). For locating the transitions state for O transfer, the potential energy surface of the reaction system was monitored while scanning the distance between the O atom in Met-OO and the S atom of Met from 3.5 Å to 1.5 Å at a decrement of 0.05 Å each step. An activation barrier was located when MetH-OO and Met was 2.4 Å apart. At this activation barrier, the bond
rupture occurs at O-O, and an O atom of Met-OO was transferred to sulfur atom of the approaching Met, accompanied by H shuttling back to amino group. The activation barrier lies slightly higher than the reactants (0.28 eV), but is lower than the barrier for the reaction of [Met - H']-OO + [Met - H'] (see section 5.3.3).

Figure 5.8 Reaction potential energy surface for Met + ^1O_2 → Met-OO, and for Met-OO + Met → 2 Met-O in acidic solution. Energies of complexes, TSs, and products were obtained from relaxed PES scan at B3LYP/6-31+G*, including solvation effects simulated by the PCM mode at 298 K.

To further complete the exploring of the pH dependence of reaction between Met and ^1O_2, we have also mapped out the reaction potential surface while methionine is protonated in acidic solution using PCM model to simulate solvent effect, as illustrated in Figure 5.9. The reaction proceeds via the similar mechanism as deprotonated and neutral Met, i.e., the formation of
persulfoxide MetH\(^+\)-OO is caused by encounter of MetH\(^+\) and \(^1\)O\(_2\), which followed by transferring one O atom to the second MetH\(^+\) molecule via an activation barrier and then resulted in the formation of MetH\(^+\)-O. The activation barrier stays 0.25 eV with respect to MetH\(^+\)-OO + \(^1\)O\(_2\).

Figure 5.9 Reaction potential energy surface for MetH\(^+\) + \(^1\)O\(_2\) $\rightarrow$ MetH\(^+\)-OO, and for MetH\(^+\)-OO + MetH\(^+\) $\rightarrow$ 2 MetH\(^+\)-O in acidic solution. Energies of complexes, TSs, and products were obtained from relaxed PES scan at B3LYP/6-31+G*, including solvation effects simulated by the PCM model at 298 K.

5.5 Comparison with photooxidation results

Before I conclude my thesis research, it is useful to review the literature results on oxidation kinetics of methionine in solution, albeit that almost all of them were performed using sensitizing
methods. The literature has reported kinetic measurements on the quenching of $^1$O$_2$ by methionine in aqueous solution. Matheson and Lee$^{11}$ reported both the reactive rate constant $k_r$ and the total quenching rate constant $k_t$ of $^1$O$_2$ with methionine, where $^1$O$_2$ was generated in D$_2$O by direct excitation of O$_2$ ($X^3\Sigma_g^-$) to $^1$O$_2$. The value of $k_t$ was evaluated by the competitive inhibition of the chemical reaction of bilirubin with $^1$O$_2$, and that of $k_r$ (loss of methionine) was measured using an amino acid analyzer. The values of $k_r$ and $k_t$ are $1.6 \times 10^7$ and $1.7 \times 10^7$ M$^{-1}$s$^{-1}$ (at pH = 8.4), respectively. Most of other reported kinetic measurements were, on the other hand, based on the dye-sensitized photooxidations. Kraljic and Sharpatyi$^{12}$ reported the $k_r$ of $2.0 \times 10^7$ M$^{-1}$s$^{-1}$ in neutral water, according to the consumption of O$_2$ in the steady state photosensitization. Lindig and Rodgers$^{13}$ examined the $k_t$ in neat D$_2$O using a laser flash photolysis method. In the experiment, the quenching of sensitization-generated $^1$O$_2$ lay in the competition for $^1$O$_2$ between the monitoring compound APDA and methionine. By holding the [APDA] constant and varying the [Met] in the photolysis, the $k_t$ was measured to be $1.5 \times 10^7$ M$^{-1}$s$^{-1}$. All these values are in agreement with those published by Miskoski and Garcia.$^{14}$ In the experiment of the latter, $^1$O$_2$ was generated by rose bengel and tryptophan was used as a sacrificial substrate. The solution was photolyzed, and the consumption of tryptophan was monitored by its fluorescence in the absence and presence of methionine. Through a Stern-Volmer treatment of the tryptophan fluorescence data, a $k_t$ value of $2.1 \times 10^7$ M$^{-1}$s$^{-1}$ was obtained for methionine at pH 7. They also measured the $k_r$ value based on the O$_2$ consumption vs. irradiation time in a solution containing sensitizer and methionine, and the $^1$O$_2$ yield of the sensitizer was calibrated using a reference compound. A unity ratio was found for $k_t : k_r$. Based on a similar Stern-Volmer approach and using diphenylfuran (DPF) as the fluorescence probe, Sysak et al. reported a $k_t$ of $1.15 \times 10^7$ M$^{-1}$s$^{-1}$. All reported value of $k_t$ are close to those of $k_r$. 

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indicating that the quenching is purely reactive in neutral aqueous solution, and there is no evidence for physical quenching.

Compared to the neutral solution, much less kinetic data was reported in basic solution, and the results are quite sensitizing dyes and reaction conditions dependent. Earlier work on pH dependence of methylene blue-sensitized photooxidation of methionine were presented by Weil and Spikes et al. in terms of the oxygen uptake, which shows remarkable increase of the O\textsubscript{2} consumption at a high pH range starting from 8. However, the reaction between excited methylene blue and methionine were involved as illustrated by the photodehydrogenenation of methionine under an anaerobic condition, and the observed pH dependence might reflect the oxidation-reduction potential between the amino acid and the excited dye. General speaking, a similar total quenching rate might be found at high pH, but a significant proportion of which would be purely physical.

Regarding the methionine photooxidation product distribution, a comprehensive work might be found in the work by Sysak et al. and Frimer. The reaction is initialized by formation of an intermediate persulfoxide, followed by the secondary reactions that are pH-dependent. At high pH (above 9), the reaction involves attack of OH\textsuperscript{-} at the sulfur atom of persulfoxide. The displacement reaction gives one molecule of sulfoxide and one molecule of H\textsubscript{2}O\textsubscript{2}. At the intermediate pH range of 6 - 10, when methionine carries a free amino group, the path leading to dehydromethionine via internal displacement becomes dominant. The structure of dehydromethionine was assigned as a five-membered heterocyclic N-S compound. H\textsubscript{2}O\textsubscript{2} is also produced in this process. Dehydromethionine may slow hydrolyze to methionine sulfoxide. At pH below 6, the persulfoxide intermediate oxidizes a second methionine molecule by the sulfide trapping mechanism, resulting in a stoichiometry of 2Met + \textsuperscript{1}O\textsubscript{2} → 2methionine.
sulfoxide (MetO).\textsuperscript{20-22} Competition among the secondary reactions accounts for the variation in O\textsubscript{2} uptake observed in solution-phase experiments, \textit{i.e.}, methionine to \textsuperscript{1}O\textsubscript{2} ratio is 0.85:1 above pH 8 and 2:1 below pH 5.\textsuperscript{15,17}

Formation of dehydromethionine and H\textsubscript{2}O\textsubscript{2} requires a free amino group basic enough to nucleophilically attack sulfur, and therefore could not occur with protonated methionine.\textsuperscript{15} In fact, the measured contribution of dehydromethionine became negligible at pH lower than $\rho$K\textsubscript{a} (= 9.2) of the ammonium group. Since the -NH\textsubscript{2} group is transformed into a cyclic =NH group in the dehydromethionine, the formation of dehydromethionine was usually determined based on continuous -NH\textsubscript{2} loss during the photooxidation according to fluorogenic detection. It is worth mentioning that the loss of primary -NH\textsubscript{2} reactivity in photooxidation depended on the dye\textsuperscript{23} and buffer ions.\textsuperscript{15} For example, little change in the -NH\textsubscript{2} reactivity was observed when eosin rather than methylene blue was used under the same conditions.\textsuperscript{23} It might be the excited dye that abstracted hydrogen atom from methionine to form dehydromethionine,\textsuperscript{15,16} and H\textsubscript{2}O\textsubscript{2} might also be formed in methylene blue radical-mediated photooxidation of methionine,\textsuperscript{16} indicating that the dehydromethionine and H\textsubscript{2}O\textsubscript{2} formation might not completely \textsuperscript{1}O\textsubscript{2}-specific.\textsuperscript{15}

The uncertainty in the above photooxidation experiments remains as to whether the oxygen consumption is completely due to the singlet oxygen-induced oxidation (\textit{i.e.}, a part of the O\textsubscript{2} consumption was caused by the generation and reactions of O\textsubscript{2}\textsuperscript{-} with substrate via Type I photosensitization\textsuperscript{15}), and the accuracy in the calibration of the photosensitization quantum yield of the sensitizers. The situation was further complicated by the reactions of methionine with excited sensitizers. The present experiment has provided a more direct monitoring technique for methionine oxidation products in aqueous system, and thus a more reliable approach for determination of absolute rate constants for \textsuperscript{1}O\textsubscript{2}-induced oxidation reactions.
5.6 Conclusions

A novel solution-phase reaction setup was successfully developed to couple with our home-built ESI-MS, which enables us to observe real-time reaction kinetics in aqueous solution under reduced pressure. The sample transfer time was reduced under 20s and enables us to probe the reaction kinetics. A demonstration of such innovative on-line reaction monitoring system is to study the reaction between methionine (Met) and short-lived single oxygen (\(^{1}\text{O}_2\)). In basic solution, \([\text{Met} - \text{H}]^-\text{O}\) and \([\text{Met} - \text{H}]^-\text{O}-\text{Met}\) were confirmed to be major products by MS experiment. An important intermediated persulfoxide was also observed. The reaction to form \([\text{Met} - \text{H}]^-\text{O}\) follows second-order kinetics and the rate constant was calculated to be \(2 \times 10^9 \text{ M}^{-2} \cdot \text{s}^{-1}\). \([\text{Met} - \text{H}]^-\text{O}-\text{Met}\) is a hydrogen-bonded complex due to the interaction between \([\text{Met} - \text{H}]^-\text{O}\) and Met. Its formation rate constant (1.4 M\(^{-1}\cdot\text{s}^{-1}\)) was derived using derivation method. At acidic solution Met-O was the only product specific to \(^{1}\text{O}_2\) reaction with Met. The rate constant is reported to be \(6 \times 10^9 \text{ M}^{-2} \cdot \text{s}^{-1}\) and follows second-order kinetics. The reaction mechanism and pH difference were elucidated with assistance from DFT calculations using polarized continuum model (PCM) to simulate bulk solvation effect. In addition, a method to determine dissolved \(^{1}\text{O}_2\) concentration in aqueous solution by correlating with gas-phase \(^{1}\text{O}_2\) emission at 1270 nm has also been developed. This approach involved on-line monitoring of UV-absorption decrease of selected \(^{1}\text{O}_2\) chemical probes. In combination with our previous results from gas-phase reaction of \(^{1}\text{O}_2\) and dry/hydrated Met (\(\text{MetH}^+(\text{H}_2\text{O})_{n=0-2}\) and \([\text{Met} - \text{H}]^+(\text{H}_2\text{O})_{n=0-2}\)), an in-depth systematic understanding of \(^{1}\text{O}_2\) mediated oxidation of Met has been established.
References

Chapter 6

Conclusions

6.1 The origin of effects on ionization states and pH dependence

Table 6.1 Summary of results from $^1$O$_2$ oxidation of gas-phase dehydrated MetH$^+$ and [Met - H], hydrated MetH$^+$(H$_2$O)$_{1,2}$ and [Met - H](H$_2$O)$_{1,2}$, as well as Met in acidic and basic solution.

<table>
<thead>
<tr>
<th>$E_{\text{col}}$ (eV)</th>
<th>Efficiency (%)</th>
<th>Precursor</th>
<th>$H_2O_2$ elimination path</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dehydrates</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MetH</td>
<td>[Met - H]</td>
<td>MetH$^+$</td>
<td>[Met - H]$^+$</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>38.0</td>
<td>0</td>
<td>sandwiched 'NH$_3$⋯OO⋯S'</td>
<td>terminal H$_3$CS⋯OO from -SCH$_3$ and -NH$_3$ from SCH$_3$ and γ-CH$_2$ [Met-2H]$^+$ + H$_2$O$_2$ none</td>
</tr>
<tr>
<td>0.2</td>
<td>48.7</td>
<td>0.8</td>
<td>sandwiched 'NH$_3$⋯OO⋯S'</td>
<td>terminal H$_3$CS⋯OO from -SCH$_3$ and -NH$_3$ from SCH$_3$ and γ-CH$_2$ 1. [Met-2H]$^+$ + H$_2$O$_2$ +H$_2$O 2. MetOOH$^+$ +H$_2$O</td>
</tr>
<tr>
<td><strong>Monohydrates</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MetH$^+$ (H$_2$O)</td>
<td>[Met - H] (H$_2$O)</td>
<td>MetH$^+$ (H$_2$O)</td>
<td>[Met - H] (H$_2$O)</td>
<td>MetH$^+$ (H$_2$O)</td>
</tr>
<tr>
<td>0.1</td>
<td>34.0</td>
<td>1.7</td>
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<td></td>
</tr>
<tr>
<td>0.2</td>
<td>7.9</td>
<td>0.8</td>
<td>sandwiched 'NH$_3$⋯OO⋯S'</td>
<td>terminal H$_3$CS⋯OO from -SCH$_3$ and -NH$_3$ from SCH$_3$ and γ-CH$_2$ 1. [Met-H]-OOH$^+$ + H$_2$O</td>
</tr>
<tr>
<td><strong>Dihydrates</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MetH$^+$ (H$_2$O)$_2$</td>
<td>[Met - H] (H$_2$O)$_2$</td>
<td>MetH$^+$ (H$_2$O)$_2$</td>
<td>[Met - H] (H$_2$O)$_2$</td>
<td>MetH$^+$ (H$_2$O)$_2$</td>
</tr>
<tr>
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<td>64.5</td>
<td>1.8</td>
<td>sandwiched 'NH$_3$⋯OO⋯S'</td>
<td>terminal H$_3$CS⋯OO 1. MetOOH$^+$ (H$_2$O) + H$_2$O 2. MetOOH$^+$ + 2H$_2$O [Met - H]-OOH (H$_2$O)$_2$</td>
</tr>
<tr>
<td>0.2</td>
<td>13.1</td>
<td>0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Aqueous solution</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reaction rate constant $k_1$ (M$^{-1}$.s$^{-1}$)</td>
<td>Intermediates</td>
<td>Products</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH = 3.2</td>
<td>10.4</td>
<td></td>
<td>MetH-OO</td>
<td>[Met - H]-OO and dimeric product [Met - H]-O-Met</td>
</tr>
<tr>
<td>6.10$^8$</td>
<td>2.10$^8$</td>
<td></td>
<td>MetH-O</td>
<td></td>
</tr>
<tr>
<td>pH = 3.2</td>
<td>10.4</td>
<td></td>
<td>[Met - H]-OO</td>
<td></td>
</tr>
<tr>
<td>pH = 3.2</td>
<td>10.4</td>
<td></td>
<td>MetH-O</td>
<td></td>
</tr>
</tbody>
</table>

The gas-phase reactions of bare/hydrated Met with $^1$O$_2$ are mediated by different types of precursor complexes for different ionization states, *i.e.*, a sandwiched $^+$NH$_3$⋯OO⋯S structure for protonated Met and a terminal H$_3$CS⋯OO structure for deprotonated Met.
In $^1$NH$_3$···OO···S, the O$_2$ moiety is sandwiched between the ammonium group and sulfur atom of the MetH$^+$. The intramolecular H transfer of -NH$_3$ to O$_2$ can proceed readily without activation barrier. Nevertheless, the interaction between an excess proton and O$_2$ is absent in deprotoned Met due to the structure of -NH$_2$. As a result, the O$_2$ moiety swings away from the amino group and is bounded to the -SCH$_3$ terminal of the [Met - H]$^-$ in the precursor.

The structural difference in the precursor complexes directly influences the subsequent reaction pathways leading to products. Both $^+$NH$_3$···OO···S and H$_3$CS···OO precursors can interconvert to hydroperoxide species. Formation of hydroperoxide from the protonated $^+$NH$_3$···OO···S precursor occurs via abstracting a H from the ammonium group; on the other hand, the H$_3$CS···OO precursor for deprotonated Met has to take the H either from the -SCH$_3$ to give rise to [Met - H]-OOH$^-$, or from the γ-C of amino acid backbone to form [Met - H]-OOH$^-_2$. This difference continuously affects the downstream reaction routes. In the reaction of protonated Met (dry and hydrated), a second proton can be simultaneously transferred from the adjacent -SCH$_3$, which opens an H$_2$O$_2$ elimination channel. The corresponding activation barriers are -0.77 and -0.11 eV for MetH$^+$ and MetH$^+(H_2O)$ with respect to reactants, respectively. In contrast, the transfer of the second H from Met to the O$_2$ is much more difficult in its deprotonated form. In order to eliminate H$_2$O$_2$, the other H has to be abstracted from the γ-C of [Met - H]-OOH$^-_1$, or from -SCH$_3$ site of [Met - H]-OOH$^-_2$. As a result, higher transition states corresponding to such H transfer are expected, which are 0.62/0.37 eV for dry [Met - H]$^-$ and 0.63/0.41 eV for [Met - H]$^-(H_2O)$ above the reactants, respectively, suggesting H$_2$O$_2$ elimination is not energetically favored in our $E_{col}$ range. Consequently, no reaction product was observed from the reaction of [Met - H]$^-$ + $^1$O$_2$, and only hydroperoxide formation takes place in the $^1$O$_2$-oxidation of [Met - H]$^-(H_2O)_{1,2}$. 

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The significantly higher reaction efficiency of hydrated Met$^+$H$_2$O$_{1,2}$ + $^1$O$_2$ compared to that of [Met - H](H$_2$O)$_{1,2}$ can also be traced back to the conformational difference in the precursor complexes. In order to form hydroperoxide [Met - H]-OOH(H$_2$O)$_{1,2}$ and 2, the precursor has to cross an 0.32/0.21 eV energy barrier at TS1(H$_2$O) and TS3(H$_2$O), respectively. In contrast, no energy barrier is needed in the hydroperoxide formation channel for the reaction of MetH$^+$H$_2$O$_{1,2}$ + $^1$O$_2$.

The effects of Met ionization states in the gas-phase reactions of methionine and $^1$O$_2$ is related to the pH dependence of reaction rate in aqueous solution. The computational study assisted us to clarify the insights of this pH dependence. As illustrated in Figures 5.7, 5.8 and 5.9, the energy barrier of transferring one O atom from persilfoxide to the second methionine molecule is 0.40 eV, 0.28 eV and 0.25 eV for methionine in deprotonated, neutral and protonated forms, respectively. The higher energy barrier in basic solution leads to the slower reaction rates, which in the meantime, allows us to capture the persulfoxide intermediate by MS measurements.

6.2 The evolution of $^1$O$_2$-mediated Met oxidation

The parallel study of $^1$O$_2$-mediated Met oxidation in the gas phase and in aqueous solution reaction has helped us to evaluate the evolution of Met oxidation dynamics and kinetics from the gas phase, through micro-solvated system, and to the aqueous solution. The reactions of $^1$O$_2$ and methionine in all systems are mediated by hydroperoxides intermediates, which carry high internal excitation and thus are very reactive. In the gas phase, the fate of the hydroperoxides is determined by whether they have exit product channel or not. In the case of protonated methionine, the transfer of two H from MetH$^+$ to O$_2$ provides an energetically favored dissociation path for hydroperoxides, which results in the elimination of H$_2$O$_2$. To the contrary,
such H$_2$O$_2$ elimination channel is inhibited by associated tight activation barriers in the reaction of deprotonated Met with $^1$O$_2$. Therefore, "hot" hydroperoxide could not convert to any stable end-products but only decay back to reactants. The fates of hydroperoxides have been altered once the system became hydrated. Since the dissociation of water ligand provides an energy dispense path to release the heat gained from formation of hydroperoxides, it enables the capture of the "relaxed" hydroperoxide species by MS measurements. As a result, hydroperoxides were detected as stable end-products in the reaction of $^1$O$_2$ with both protonated and deprotonated hydrated Met. Compared to the gas-phase single molecular collision, highly reactive hydroperoxides once formed in solution readily react with another molecule of Met, leading to the formation of methionine sulfoxide as final product.