Mechanistic and kinetic study of singlet O$_2$ oxidation of methionine

by on-line electrospray ionization mass spectrometry

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Abstract. We report a reaction apparatus developed to monitor singlet oxygen (1O$_2$) reactions in solution using on-line ESI mass spectrometry and spectroscopy measurements. 1O$_2$ was generated in the gas phase by the reaction of H$_2$O$_2$ with Cl$_2$, detected by its emission at 1270 nm, and bubbled into aqueous solution continuously. 1O$_2$ concentrations in solution were linearly related to the emission intensities of airborne 1O$_2$, and their absolute scales were established based on a calibration using 9,10-anthracene dipropionate dianion as an 1O$_2$ trapping agent. Products from 1O$_2$ oxidation were monitored by UV-Vis absorption and positive/negative ESI mass spectra, and product structures were elucidated using collision-induced dissociation-tandem mass spectrometry. To suppress electrical discharge in negative ESI of aqueous solution, methanol was added to electrospray via in-spray solution mixing using theta-glass ESI emitters. Capitalizing on this apparatus, the reaction of 1O$_2$ with methionine was investigated. We have identified methionine oxidation intermediates and products at different pH, and measured reaction rate constants. 1O$_2$ oxidation of methionine is mediated by persulfoxide in both acidic and basic solutions. Persulfoxide continues to react with another methionine, yielding methionine sulfoxide as end-product albeit with a much lower reaction rate in basic solution. Density functional theory was used to explore reaction potential energy surfaces and establish kinetic models, with solvation effects simulated using the polarized continuum model. Combined with our previous study of gas-phase methionine ions with 1O$_2$, evolution of methionine oxidation pathways at different ionization states and in different media is described.

Key words: On-line ESI mass spectrometry, Reaction kinetics, Methionine, Singlet oxygen, Persulfoxide, Sulfoxide, Photooxidation, DFT-PCM calculation

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Introduction

Methionine (Met) is one of the five amino acids (the other four are tryptophan, tyrosine, histidine and cysteine) that are most susceptible to the attack by reactive oxygen species (ROS) [1-3]. Oxidation of Met residues in proteins produces sulfoxides (MetO) [4-6]. Under extremely strong oxidative conditions MetO can be irreversibly oxidized to sulfone (MetOSO) [3, 6]. It is well accepted that hydrophobic contacts via the Met residues contribute to protein stability. Moreover, Met has the propensity to interact with aromatic residues, and the resulting Met sulfur-aromatic motif provides additional stabilization over hydrophobic interactions [7]. Oxidation of Met to MetO decreases hydrophobicity [5] and disrupts both dispersion and electrostatic interactions present in the sulfur-aromatic motif [7]. Such oxidation-induced post-translational modifications may cause proteins to change conformations and lose functions [8-10], and are related to pathophysiological conditions such as cancer, aging, and neurodegenerative diseases [3, 11]. On the other hand, surface-exposed Met residues act as endogenous antioxidants or "molecular bodyguards" under oxidative stress, protecting other residues important to the functions of proteins [12, 13]. This is due to the fact that cells are capable of reducing MetO back to Met, catalyzed by Met sulfoxide reductases (Msrs) [14-16]. The reversible Met oxidation is also identified as a trigger for the functions of two protein kinases CaMKII and transcription factor HypT (HOCl specific) [17, 18]. Consequently, the contents of Met and MetO in cells can be used to indicate the extent of ROS generation, and the activities of oxidant scavengers and Msrs.

Oxidation kinetics of Met to MetO has been investigated in the presence of various ROSs [3, 19-24], of which $^1$O$_2$-mediated kinetics was mostly measured using photooxidation methods [23, 24]. In these experiments, $^1$O$_2$ was generated via energy transfer from the triplet excited state of a sensitizer to $^3$O$_2$ (type II photosensitization) [25]. Photooxidation of Met [24, 26-32] depends on various parameters (e.g., pH, O$_2$ concentration, solvent composition, buffer ions, and combination of light and sensitizers). Determination of $^1$O$_2$-mediated oxidation kinetics was also challenged by the competition between radical- and $^1$O$_2$-mediated reactions, and the interaction between substrates and excited sensitizers.
To avoid the interferences in photooxidation experiments, one approach is to use heterogeneous photosensitization in which sensitizers are isolated from reaction solution and the sensitizer-generated 1O2 is delivered through space [33] or via bubbles [34]. We have adopted another approach based on a chemical reaction to produce radical-free, "clean" 1O2. Coupled with ion-beam-scattering methods and electrospray ionization mass spectrometry (ESI MS), we have investigated 1O2-mediated oxidation dynamics of Met in the gas phase at different ionization and hydration states (including [Met + H]+(H2O)0-2 and [Met - H](H2O)0-2) [35, 36]. Study of the 1O2 reactions with dehydrated [Met + H]+/[Met - H] has allowed us to identify the intrinsic interactions between Met and 1O2; and that of the 1O2 reactions with hydrated [Met + H]+(H2O)1-2/[Met - H](H2O)1-2 has enabled us to recognize the effects of individual solvent molecules. On the one hand, gas-phase findings provide fundamentals in understanding the oxidation mechanism; on the other hand, they suffice as valuable references in examining specific and continuum solvent effects, and it will be interesting to see how the gas-phase findings can be extrapolated to solution models. This prompted us to look at 1O2 oxidation of Met in aqueous solution, aimed at investigating the resemblance and evolution of reaction pathways and products from the gas phase to aqueous continuum.

Over recent years, applications of ESI MS have spread rapidly in monitoring solution-phase reaction kinetics and probing the course of chemical transformations [37-39]. The fast growth of such applications relies on several exclusive advantages of ESI MS: 1) The ESI process is simple, "clean" and "soft" where no harsh conditions are applied to the sample solution being transferred into mass spectrometer. As a result, weakly bound species may be detected in the gas phase; 2) On-line ESI MS is a rapid and sensitive approach that detects multiple products simultaneously at very low concentrations (nanomolar), and provides chances for capturing short-lived intermediates which might not survive the normal handling for off-line analysis; 3) Each mass spectrum represents a snapshot of an on-going reaction, and kinetics can be measured through continuous analyses of the reaction mixture.

However, monitoring 1O2 reaction kinetics in solution puts severe demands on an on-line ESI MS
experiment, which warrant careful consideration in the design of the corresponding apparatus and methodologies. First, the reaction apparatus is continuously pumped in order to replace quenched O$_2$ in solution with fresh $^1$O$_2$; consequently, ESI MS has to sample solution from a low-pressure reaction system. Secondly, we need to record ESI MS of aqueous sample in both positive and negative modes. Note that water has a high surface tension that requires high ESI spray potentials. The high spray potentials may lead to the presence of conventional corona discharges at the ESI emitter. For otherwise equivalent conditions a breakdown due to a corona discharge occurs at significantly lower field strength in the case of the negative polarity than in the case of the positive one, and the ESI operation becomes impossible without discharge suppression [40, 41]. However, the method to be used for discharge suppression should pose inappreciable interference with the aqueous sample. Thirdly, the sample transfer time must be minimized so that the reaction progress can be tracked promptly. To meet these specifications, a new on-line ESI MS and spectroscopy monitoring system was developed in the present work. Capitalizing on this apparatus, we have measured the reaction kinetics between Met and $^1$O$_2$ in different pH solutions. The results were interpreted with the aid of electronic structure calculations. Combined with the recently reported gas-phase dynamics of bare/hydrated Met ions with $^1$O$_2$ [35, 36], a sound understanding has been obtained for Met oxidation.

**Experimental and computational details**

$^1$O$_2$ generation, detection and reaction

$^1$O$_2$ was generated by the reaction of H$_2$O$_2$ + Cl$_2$ + 2KOH $\rightarrow$ O$_2$ ($\sim 85\% X^3\Sigma_g^-$ and $\sim 15\% a^1\Delta_g$) + 2KCl + 2H$_2$O [35, 42, 43]. As shown in Fig. 1, 13 mL of 8 M KOH was added to 20 mL of 35 wt% aqueous H$_2$O$_2$ in a sparger (1) which was immersed in a chiller held at -19 °C. The resulting mixture was degassed quickly. 2.6 sccm of Cl$_2$ ($\geq 99.5\%$, Sigma-Aldrich) was mixed with 96 sccm of He and bubbled through the H$_2$O$_2$/KOH slush. All of the Cl$_2$ reacted with H$_2$O$_2$. The gaseous products passed through a cold trap (2, kept at -70 °C) to remove water vapor. Only $^1$O$_2$, $^3$O$_2$ and He remained in the downstream gas. The concentration of $^1$O$_2$ in the gas was determined by measuring $^1$O$_2$ emission ($a^1\Delta_g \rightarrow X^3\Sigma_g^-$, $\nu = 0$
– 0) \cite{44} at 1270 nm in an optical emission cell (3). Emission from the cell was collected using a plano-convex lens, passed through an optical chopper (SRS model SR540) and 5-nm bandwidth interference filter centered at 1270 nm, and focused by another plano-convex lens into a thermoelectrically cooled InGaAs detector (4, Newport model 71887) coupled with a lock-in amplifier (SRS model SR830). \(^1\)O\(_2\) (mixed with \(^3\)O\(_2\) and He) was then bubbled into the aqueous solution in a reaction vessel (5). During the experiment the entire apparatus was continuously pumped with a mechanical pump, and the pressure of the apparatus was maintained at 25 \(\tau\) (slightly above water vapor pressure at room temperature) through a pressure relay (6, Cole-Parmer 00244OW). The pumping serves several purposes: reducing the residence time of \(^1\)O\(_2\) in the gas phase and therefore minimizing its wall quenching and self-quenching, and removing quenched O\(_2\) and replenishing fresh \(^1\)O\(_2\) to the reaction solution. Since a significant amount of water evaporated from 5 at the low operating pressure, extra water was replenished into 5 through an Ismatec Reglo-CPF rotary piston pump (7) at a precisely controlled flow rate.

To check the reactivity of aqueous substrates towards \(^3\)O\(_2\)/He, control experiments were performed under the same conditions as those for \(^1\)O\(_2\), except that Cl\(_2\) was replaced by O\(_2\) gas at the same flow rate.

**On-line sampling for spectroscopy and ESI MS**

Our monitoring system includes a UV-Vis spectrometer and an ESI tandem mass spectrometer. Absorption and mass spectra were recorded over the course of reactions. As illustrated in Fig. 1, the aqueous reaction solution was circulated by a peristaltic pump (8) through a quartz flow cell (9, Starna Cells 583.65-Q-10/Z15, 1-cm path length). UV-Vis absorption was monitored using an Ocean Optics USB4000 diode array spectrometer (10). Spectra were recorded at 30-s intervals using the Ocean Optics SpectraSuite software.

To the best of our knowledge, most on-line ESI MS approaches sampled reaction solution at a pressure near atmospheric or higher, where the solution was delivered to ESI by gravity or positive gas pressure \cite{45-51}. However, our reaction system needs to be maintained at a pressure of 25 \(\tau\) as rationalized above. Therefore, one feature of our sampling system is to transport sample from low-
pressure solution to open-air ESI, using a sampling loop (0.035 mL vol.) coupled to a 2-position switching valve (11). The valve was controlled by a LabVIEW program. For each measurement, the valve was placed in load position for 2 s to fill the loop with reaction solution through 8, and then switched to injection position for transferring the solution to electrospray. The time lag between sample load and MS measurement is determined by the dead volume (~0.1 mL) of the transfer line connecting the sampling loop to ESI, and the sample transfer rate. Note that a typical flow rate for ESI is 1 µL/min, which would result in far too long delay for kinetic measurements if this rate is used for sample transfer.

In a previous on-line ESI MS experiment [52], we adopted Cooks and coworkers’ method [51] by adding a splitter to the sample transfer line, right before ESI. The sample was swept to ESI under N₂ gas pressure. This configuration allowed for a high sample transfer rate (11.4 mL/hr), and in the meantime delivered only a small fraction of the flow to ESI. We were able to reduce the sample transfer time to less than 30 s [52]. But this method increased the total sample volume needed (by a factor of ~ 200) for each measurement. In the present work, we developed another approach to reduce the lag time. The sampling loop was swept by water plug using a syringe pump (12a) at programmed flow rates. For each sampling, the flow rate of 12a was first set to 6 mL/hr. During this duration, the sample was loaded, inserted into the transfer line and transferred to the ESI emitter. After that, the rate of 12a decreased to 0.04 mL/h until the completion of MS measurement. In the positive ion mode, the ESI emitter was assembled by gluing the stainless steel hypodermic tubing (35-gauge, 0.005” o.d. × 0.002” i.d. × 0.5” length, Small Parts Inc.) to the exit (PEEK tubing, 0.0625” o.d. × 0.007” i.d.) of 11, and the total time for sample load and transfer was minimized to less than 10 s.

An issue for negative ESI of aqueous solution is electrical discharge. Due to the high surface tension of water, its ESI onset field strengths are ±8.9 × 10⁶ V/m in positive and negative ion modes, respectively (much higher than those of ±5 × 10⁶ V/m for methanol) [41]. Unfortunately, for the negative ion mode corona discharge at the stainless steel tip occurred at lower field strength than the ESI onset [41]. As a result, the stainless steel emitter could not be used in negative ESI. Cassou et al. reported negative nano-
ESI of water solution by using borosilicate capillary with an ESI spray potential of -0.7 kV and a 3-mm distance between the emitter and the counter electrode [53]. We were not able to achieve stable ESI with the same field strength, presumably due to the higher flow rate we used. Our approach is to utilize a borosilicate theta-glass capillary (13, Sutter Instrument) as an ESI emitter [54-56], and mix aqueous sample solution and methanol at the tip of the emitter so as to lower ESI operating potential. Aqueous solution and methanol were individually delivered by syringe pumps 12a and 12b at a flow rate 0.01 and 0.03 mL/h, respectively, and directed into the two separated channels within the theta capillary through short PEEK tubing (14a and 14b, 255 µm i.d. × 510 µm o.d.). The theta capillary was laser-pulled to a tip size of 20 µm i.d. × 40 µm o.d. by a micropipette puller (Sutter Instrument P-2000). A PEEK microtee (15, Upchurch Scientific, 0.1 µL dead volume) was inserted to the aqueous sample line, and a platinum wire (16, 0.005" o.d., Alfa Aesar) was inserted into one stem of 15 to supply the electrical connection (-2.8 kV) for ESI. In this in-spray solution mixing, the encounter of aqueous solution and methanol took place at the time when solution reaction was terminated by spray. Consequently, interference with aqueous reactions by methanol was minimized, and diffusion and turbulence were avoided during fluid mixing.

**ESI MS and collision-induced dissociation-tandem MS measurements**

A home-built guided-ion-beam tandem mass spectrometer was used for ESI MS measurements. The operation, calibration and data analysis procedures for the mass spectrometer were described previously [57]. In brief, the apparatus consists of an ion source, radio frequency (rf) hexapole ion guide, quadrupole mass filter, rf octopole ion guide surrounded by a scattering cell, second quadrupole mass filter, and a pulse-counting detector. Both quadrupole mass filters use Extrel 9.5 mm tri-filter rods and were operated at 2.1 MHz with a detectable mass/charge (m/z) range of 1 - 500.

An ESI emitter was held at 3.7 and -2.8 kV, respectively, for producing positively and negatively charged species from sample solution. Charged droplets entered the source chamber of the mass spectrometer through a desolvation capillary (which was heated to 130 °C and held at 100 V for positive
ions and -120 V for negative ones). The distance between the emitter tip and the entrance of the desolvation capillary was 1 cm. Liquid droplets underwent desolvation as they passed through the heated capillary, converted to gas-phase ions in the source chamber. A skimmer with an orifice of 0.99 mm is located 3 mm from the capillary end, separating the source chamber and the hexapole ion guide. The skimmer was biased at 20 V for positive ions and -20 V for negative ones. Ions were transported into the hexapole at a pressure of 26 m\(\tau\), undergoing collisional focusing and cooling to 310 K. In conventional ESI MS measurements, the first quadrupole mass filter was rendered to an rf-only ion guide, and ions were mass-analyzed by the second quadrupole.

In order to identify the structures of product ions, collision-induced dissociation (CID) was performed. The product ions of interest were mass-selected by the first quadrupole, and then injected into the octopole ion guide which trapped ions in the radial direction. DC bias voltage was applied to the octopole, allowing control of the kinetic energy \(E_{\text{lab}}\) of ions in the laboratory frame. \(E_{\text{lab}}\) can be converted to the collision energy \(E_{\text{col}}\) between ions and collision gas in the center-of-mass frame using
\[
E_{\text{col}} = E_{\text{lab}} \times \frac{m_{\text{neutral}}}{M_{\text{ion}} + m_{\text{neutral}}},
\]
where \(m_{\text{neutral}}\) and \(M_{\text{ion}}\) are the masses of neutral collision gas and ions, respectively [58]. The octopole runs through the scattering cell filled with Xe gas (99.995%). The pressure of the cell was controlled using a leak valve and measured by a Baratron capacitance manometer (MKS model 690 head and 670 signal conditioner). CID was measured at \(E_{\text{col}} = 0.5, 1.0\) and 1.5 eV with the cell pressure of 0.3 mTorr. Primary ions and fragment ions were collected by the octopole, and directed to the second quadrupole for mass analysis.

**Electronic structure calculations**

To aid in reaction mechanism interpretation, density functional theory (DFT) electronic structure calculations were performed using Gaussian 09 [59]. All structures were fully optimized at the B3LYP/6-31+G* level of theory. To take into account the solvation effects for aqueous reactants, transition states (TSs), intermediates and products, we have employed the polarized continuum model (PCM) [60] which creates solute cavity via a set of overlapping spheres in the DFT calculations for all species. The DFT-
PCM method has been successfully used for probing and reproducing the solution-phase chemistry of $^{1}\text{O}_2$ and peroxides, for example the $^{1}\text{O}_2$ oxidation of 6-thioguanine [61] and the transformation of 8-oxoguanine [62] in water. Conformation searching was conducted for all reactants, and their most stable confirmations were used as starting geometries in construction of reaction potential energy surfaces (PESs). All of the TSs were verified as first-order saddle points, and the vibrational mode with an imaginary frequency corresponds to the associated reaction pathway. All reported reaction energetics include zero-point energies (ZPEs) and thermal corrections at 298 K, with ZPEs scaled by a factor of 0.977 [63]. The energy barriers of TSs (with respect to reactants) were refined by single-point calculations at B3LYP/PCM/aug-cc-pVTZ using the B3LYP/PCM/6-31+G*-optimized reactants and TS geometries.

**Results**

**$^{1}\text{O}_2$ concentrations in solution**

In the experiment chemically generated $^{1}\text{O}_2$ was continuously bubbled into aqueous solution in the reaction vessel. $^{1}\text{O}_2$ has a longer lifetime in the interior of bubbles (due to reduced encounters with water) than in bulk solution. After diffusing into the bulk water, $^{1}\text{O}_2$ has a lifetime of $\sim 2\ \mu$s and can travel only $\sim 150\ \text{nm}$ [64]. Therefore, $^{1}\text{O}_2$ reactions occurred both at the gas/liquid interface of bubbles and in the bulk solution. Considering the steady concentration of airborne $^{1}\text{O}_2$ (as determined by the $^{1}\text{O}_2$ emission intensity which varied within 10% over the course of reaction), and the continuous feeding of fresh $^{1}\text{O}_2$ into the reaction solution, a quasi-steady-state [[$^{1}\text{O}_2$]] may be assumed for our reaction system as people did for bubbled $^{1}\text{O}_2$ in heterogeneous photosensitization [34]. In order to validate this assumption and determine the average value of [[$^{1}\text{O}_2$]] in solution, two $^{1}\text{O}_2$ chemical trappers, 9,10-anthracene dipropionate dianion (ADPA, Chemodex) and uric acid (99%, Alfa Aesar), were used as calibration compounds.

ADPA is known to react with $^{1}\text{O}_2$ chemically (i.e. without physical quenching), producing endoperoxide via [4+2] cycloaddition accompanied by bleaching of the absorption band of ADPA [65]. Fig. 2a shows absorption changes of ADPA along the reaction course. The pH of the ADPA solution
(0.05 mM) was maintained at 10.0 using borax/NaOH buffer. Shown in the insert of Fig. 2a is the plot of $\ln(A_t/A_0)$ vs. reaction time, where $A_t$ and $A_0$ are the ADPA peak absorption (at 378 nm) at different reaction times and time zero, respectively. The observation of a linear relationship between $\ln(A_t/A_0)$ and reaction time indicates that consumption of ADPA obeys first-order rate law. Accordingly, an average $[^1O_2]$ in solution can be extracted using the reaction rate $k_r$ ($8.2 \times 10^{-7}$ M$^{-1}$s$^{-1}$) for ADPA + $[^1O_2]$ [65].

During each experiment, emission of airborne $[^1O_2]$ was continuously monitored. Fig. 2b presents the correlation between the output of the emission detector and the corresponding ADPA-calibrated $[^1O_2]$ in solution. It shows that $[^1O_2]$ in solution increases linearly with the growth of airborne $[^1O_2]$ emission. The calibration curve crosses the x-axis at ~50 mV. This indicates a threshold airborne concentration below which all of the $[^1O_2]$ quenched in bubbling and diffusion before reaching aqueous substrates. In the subsequent experiments, the calibration curve of Fig. 2b was used to determine $[^1O_2]$ in solution.

We also used uric acid [66-70] as a chemical trapper to test our reaction setup, and confirmed that $[^1O_2]$-induced uric acid oxidation follows first-order kinetics (see supplementary Fig. S1 in the Supporting Information). This further supports the pseudo-steady-state assumption for $[^1O_2]$ in our solution reactions. However, the literature reported $k_r$ values for uric acid were based on bleaching of uric acid by photooxidation and did not represent exclusive chemical reactions with $[^1O_2]$. Mantana et al. [68] reported that both $[^1O_2]$-dependent and -independent mechanisms were involved in photobleaching of uric acid. Kanofsky [66] and Fischer et al. [67] found that uric acid was able to directly quench sensitizers. Rabello et al. [70] reported consecutive reaction steps for uric acid oxidation depending on sensitizers. For these reasons, the reported $k_r$ of uric acid was not used for qualitative determination of $[^1O_2]$ in our experiment.

**Met oxidation products, mechanism and kinetics in basic solution**

We present first the $[^1O_2]$ oxidation results of Met in basic solution. The reaction solution (pH = 10.4) was prepared by adding NaOH to 2.5 mM Met aqueous solution. Considering the p$K_a$ values of Met (p$K_a$ = 2.13 and p$K_b$ = 9.28) [71], deprotonated [Met - H]$^-$ is the predominant species (i.e. 93% [Met - H]$^-$/ vs. 7% neutral Met) in this solution. Major product ions were found at $m/z$ 164 and 313, corresponding to
deprotonated sulfoxide \([\text{MetO} - \text{H}]^-\) and its Met adduct \([\text{MetO} - \text{H}]^-\text{Met}\), respectively. As shown in Fig.3, the intensities of both products increase along the reaction time. Note that formation of dehydromethionine \([\text{Met} - 3\text{H}]^+\) was reported in the photooxidation of Met at pH 6 -10 [24]. But no \([\text{Met} - 3\text{H}]^+\) was observed in our mass spectra.

It is possible that the product ions of \(m/z\) 164 have contribution from doubly charged covalently bound dimer \([\text{Met} - \text{H}]^-\text{OO-}[\text{Met} - \text{H}]^+\). We have examined the isotope distribution at \(m/z\) 165, but did not observe obvious contribution from \(^{34}\text{S}\)-containing \([\text{Met} - \text{H}]^-\text{OO-}[\text{Met} - \text{H}]^+\). For further verification, we have carried out CID of the ions \(m/z\) 164 with Xe. If the ions were from \([\text{Met} - \text{H}]^-\text{OO-}[\text{Met} - \text{H}]^+\), singly charged \([\text{MetOO} - \text{H}]^-\) would be expected in CID products. However, no fragment ions were observed in the \(m/z\) range higher than 164 at \(E_{\text{col}} = 0.5 - 1.5\) eV, ruling out this possibility.

CID of the product ions \(m/z\) 313 with Xe produced two fragment ions at \(m/z\) 164 and 148, which confirms that this species is an adduct of a neutral Met molecule to \([\text{MetO} - \text{H}]^-\). Our initial guess for the structure of \([\text{MetO} - \text{H}]^-\text{Met}\) was that the Met is covalently bonded to \([\text{MetO} - \text{H}]^-\) via S(=O)-S (thiosulfinate) or S-O-S linkage [72]. A grid search was then used to find global minima in their conformation landscapes. Each of the torsion angles of Met backbones was rotated systematically through 360° at 30° increments to generate trial staggered confirmations. To our surprise, all of the trial conformations converged to hydrogen-bonded structures of \([\text{MetO} - \text{H}]^-\text{Met}\). The most stable conformer lies 48.24 kJ/mol below the reactants, as shown in Scheme 1.

**Scheme 1**  Lowest-lying conformation of hydrogen-bonded \([\text{MetO} - \text{H}]^-\text{Met}\)

Our mass spectra also revealed a trace of ion signals at \(m/z\) 180 with no obvious time dependence after its initial rising. We have attributed this species to mainly persulfoxide \([\text{MetOO} - \text{H}]^-\). Persulfoxide
is a common intermediate in the $^1\text{O}_2$ oxidation of dialkyl sulfide R$_2$S. However, persulfoxide readily
reacts with a second R$_2$S to yield sulfoxide [73]. The species observed at m/z 180 could be the remaining
[MetOO - H]$^-$ which survived on-line transfer. One complication in the interpretation of m/z 180 is that
the [Met - H]/CH$_3$OH adduct was expected at the same m/z when methanol was added in negative ESI.
To confirm the presence of [MetOO - H]$^-$, we repeated the experiment using ethanol as a supplementary
solvent for ESI. The peak of m/z 180 was still visible in product mass spectra, verifying formation of
[MetOO - H]$^-$ in Fig. 3a the contribution from [Met - H]/CH$_3$OH has been subtracted at m/z 180.

Note that an alternative structure for m/z 180 could be sulfone [MetOSO - H]$^-$ We have excluded this
possibility for several reasons. It was reported that formation of sulfone occurs to a much lesser extent in
Met oxidation [3, 6]. Based on DFT calculations, the reaction enthalpy for formation of [MetOSO - H]$^-$
is -367.6 kJ/mol with respect to [Met - H]$^-$ + $^1$O$_2$, which is more exothermic than that of [MetOO - H]$^-$.
[MetOSO - H]$^-$, if it did form in the reaction, would have been observed in product mass spectra with
considerable intensity. However, this is not the case in our measurements. The absence of sulfone could
be due to a tight and high transition state (96.5 kJ/mol above the reactants, calculated at B3LYP/PCM/631+G*) for the interconversion from [MetOO - H]$^-$ to [MetOSO - H]$^-$.

To dissect the oxidation mechanism and elucidate the intermediacy of [MetOO - H]$^-$, we have
mapped out the reaction PESs using relaxed PES scan at B3LYP/6-31+G*. Solvent effects were
simulated using the PCM model. In PES calculations, the following reaction steps were assumed
following Foote's sulfide photooxidation mechanism [74], of which the first step corresponds to
formation of a key intermediate persulfoxide, and the persulfoxide can react with another [Met - H]$^-$
leading to two sulfoxides.

\[
\begin{align*}
\text{[Met - H]$^-$ + }^1\text{O}_2 \rightarrow & \text{[MetOO - H]$^-$} \quad \Delta H_{rxn} = -62.7 \text{ kJ/mol} \quad (1) \\
\text{[MetOO - H]$^-$ + [Met - H]$^-$} \rightarrow & 2[\text{MetO - H}$^-$] \quad \Delta H_{rxn} = -245.1 \text{ kJ/mol} \quad (2)
\end{align*}
\]

In Fig. 4a the potential energy for the first step is continuously monitored while the O$_2$ moiety is
approaching the Met sulfur atom. The PES scan continuously varied the distance between S and the
"closer" oxygen atom of O₂ at a step size of 0.05 Å from 6.0 Å to 1.65 Å until a stable [MetOO - H]⁻ structure formed. All coordinates other than r(O-S) were optimized at each step. As illustrated in the PES, there are no activation barriers above reactants leading to formation of [MetOO - H]⁻. The reaction initially forms a weakly bound precursor complex with r(O-S) of 2.15 Å. This electrostatic interaction-based precursor crosses over a small barrier TS1⁻ at r(O-S) of 1.95 Å, evolving to a covalently bound product [MetOO - H]⁻ which locates 62.7 kJ/mol below the isolated reactants.

Fig. 4b shows a 2D PES for the second step reaction along the reaction coordinates r(O-S) and r(O-O). r(O-S) is the distance between the terminal O atom of [MetOO - H]⁻ and the S atom of [Met - H]⁺, and r(O-O) is the peroxide bond length of [MetOO - H]⁻. These coordinates were chosen because r(O-S) describes the approach of reactants, while r(O-O) corresponds to the separation into two [MetO - H]⁻ products. The PES was fit to nearly 700 points calculated at B3LYP/PCM/6-31+G*, and we optimized all other coordinates at each point. On this 2D surface, there is one activation barrier (TS2⁻) located at the bottleneck leading from the reactants to the product well, with r(O-O) ~ 1.69 Å, r(O-S) ~ 2.42 Å and an energy of 38.6 kJ/mol in excess of the reactants. This barrier accounts for the energy required to dissociate peroxo linkage and transfer the terminal O to the S of the approaching [Met - H]⁺. The reaction enthalpy for eq. (2) is calculated to be -245.1 kJ/mol.

Analysis of the PESs provides insight into the Met oxidation mechanism in basic solution. Particularly, it verifies eqs. (1) and (2) as two consecutive elementary steps. Both steps are bimolecular reactions, and the second one acts as the rate-limiting step due to the associated high-energy TS2⁻. We therefore conclude that formation of [MetOO - H]⁻ is much faster than its subsequent conversion to [MetO - H]⁺. Note that [MetOO - H]⁻ always presented low abundance in products. As illustrated in the product time profiles of Fig. 3, the abundance of [MetOO - H]⁻ rose from zero after the initial induction period and then had no obvious change during the major part of the reaction. Under these conditions, we made quasi-steady-state approximation (QSSA) [75] for [MetOO - H]⁻, i.e.,

\[
\frac{d[\text{[MetOO} - \text{H}]^-]}{dt} = k_1[\text{[Met} - \text{H}]^-][O_2] - k_4[\text{[MetOO} - \text{H}]^-] - k_5[\text{[MetOO} - \text{H}]^-][\text{[Met} - \text{H}]^-] = 0
\]  

(3)
where the rate constants $k_1^-$ and $k_1^-$ are for the forward and backward reactions of eq. (1), and $k_2^-$ for eq. (2). The reverse direction of eq. (1) represents a fraction of $^{1}\text{O}_2$ physical quenching that takes place via decomposition of [MetOO - H$^-$], partially accounting for the reaction inefficiency [76]. [Met - H$^-$] does not have an appropriate low-lying excited state to deactivate $^{1}\text{O}_2$ by electronic energy transfer, and a charge-transfer quenching mechanism is unlikely due to its large reaction endothermicity [36]. On the other hand, movement along the PES for [Met - H$^-$] $+$ $^{1}\text{O}_2$ $\rightarrow$ [MetOO - H$^-$] may bring the persulfoxide into a region near the rapidly rising triplet state PES where intersystem crossing could occur [76]. A similar physical quenching mechanism was observed in the gas-phase collisions of $^{1}\text{O}_2$ with hydrated and dehydrated [Met - H$^-$] [36].

Considering eq. (2) is rate-limiting, the steady state concentration of [MetOO - H$^-$] implies that a large fraction of [MetOO - H$^-$] must decay back to reactants rather than converted to [MetO - H$^-$] (i.e. $k_1^-[[\text{MetOO - H}^-]] \gg k_2^-[[\text{MetOO - H}^-]][[\text{Met - H}^-]]$). We can therefore eliminate $k_2^-[[\text{MetOO - H}^-]][[\text{Met - H}^-]]$ in eq. (3). This leads to a pre-equilibrium for eq. (1) as expressed by eq. (4), where $K^-$ is the equilibrium constant.

$$[[\text{MetOO - H}^-]] = \frac{k_1^-[[\text{Met - H}^-]'][^{1}\text{O}_2]}{k_1^-} = K^- [[\text{Met - H}^-]]'^{1}\text{O}_2$$

(4)

The rate law for formation of [MetO - H$^-$] can now be given as

$$\frac{d[[\text{MetO - H}^-]]}{dt} = k_2^-[[\text{MetOO - H}^-]][[\text{Met - H}^-]] = K^- k_2^-[[\text{Met - H}^-]]'^{1}\text{O}_2 = k^- [[\text{Met - H}^-]]'^{1}\text{O}_2$$

(5)

where $k^-$ is the effective rate constant ($M^{-2}s^{-1}$). Among the three products ([MetO - H$^-$], [MetOO - H$^-$] and [MetO - H$^-$]...Met), [MetOO - H$^-$] is minimal (< 0.1% over a period of 40 minutes) and can be safely ignored in the total product concentration. [MetO - H$^-$]...Met originates from the secondary reaction of [MetO - H$^-$], and was lumped together with [MetO - H$^-$] in calculating the total [MetO - H$^-$] concentration. As a result, eq. (5) can be approximated as

$$\frac{d[[\text{MetO - H}^-]]}{dt} = 2k^- ([[[\text{Met - H}^-]]_0 - [[\text{MetO - H}^-]])^2'^{1}\text{O}_2$$

(6)
and its integrated form as

\[
\frac{1}{[[\text{Met} - H]]_{o}} - \frac{1}{[[\text{MetO} - H]]_{t}} = 2k'\left[\text{O}_2 \right]dt
\]  

(7)

or

\[
\frac{[[\text{MetO} - H]]}{[[\text{Met} - H]]\left[\text{O}_2 \right]_{ave}} = 2k'\left[\text{Met} - H\right]_{t}
\]

(8)

At the reaction time \(t\), \(\frac{[[\text{MetO} - H]]}{[[\text{Met} - H]]\left[\text{O}_2 \right]_{ave}}\) can be measured using the relative abundances of reactant and product intensities in the corresponding mass spectrum. \(\left[\text{O}_2 \right]_{ave}\) was averaged over the integration period from 0 to \(t\). As shown in Fig. 3b, the plot of \(\frac{[[\text{MetO} - H]]}{[[\text{Met} - H]]\left[\text{O}_2 \right]_{ave}}\) vs. \(t\) fits in to a linear relationship, supporting the proposed mechanistic scheme. The measured value of \(k'\) is \(1.0 \times 10^9\) M\(^{-2}\) s\(^{-1}\).

In the above two-step scheme, formation of [MetO - H] can also be viewed as a trimolecular reaction that avoids nearly simultaneous three-body collisions [77]. Assuming \(k_2\) follows the Arrhenius equation with the activation energy \(E'_2\) equal to TS\(_2\) (38.6 kJ/mol), the overall activation energy \(E'_{a}\) for this trimolecular reaction is given by eq. (9) [77]. Since the \(\Delta H_{rxn1}\) for the first step is -62.7 kJ/mol and the activation energy for the second step is not too high, \(E'_{a}\) for this trimolecular reaction becomes negative:

\[
E'_{a} = RT^2 \frac{dlnK}{dT} + RT^2 \frac{dlnk_2}{dT} = \Delta H_{rxn1} + E'_2 = -24.12\ kJ/mol
\]

(9)

Met oxidation products, mechanism and kinetics in acidic solution

The oxidation product of Met in pH 3.2 solution (prepared by adding equimolar HCl to 0.5 mM Met aqueous solution) was detected at \(m/z\ 166\) only, and its intensity increased with the reaction time as shown in Fig. 5a. This product can be attributed to protonated [MetO + H]\(^{+}\) or [Met + H]\(^{+}\)-OO-[Met + H]\(^{+}\). We have performed CID of \(m/z\ 166\) with Xe, and expected that [Met + H]\(^{+}\)-OO-[Met + H]\(^{+}\) would lose a proton in CID. However, no [Met + H]\(^{+}\)-OO-Met was observed in CID, indicating that no [Met + H]\(^{+}\)-OO-[Met + H]\(^{+}\) was formed in the reaction.

Note that due to the low carboxyl p\(K_a\) of Met, the percentile populations of neutral and protonated Met in pH 3.2 solution are 92% and 8%, respectively. As a result, the kinetics at pH 3.2 represents the
oxidation chemistry of neutral Met (in a zwitterionic structure), albeit that the reactant and products were protonated in positive ESI spray. Therefore, the following kinetics discussion focuses on neutral Met. Similar as its deprotonated counterpart, we may propose two steps for oxidation of neutral Met. Their reaction enthalpies were calculated at the B3LYP/PCM/6-31+G* level of theory:

\[
\text{Met} + ^1\text{O}_2 \rightleftharpoons \text{MetOO} \quad \Delta H_{\text{rxn}} = -83.0 \text{ kJ/mol} \quad (10)
\]

\[
\text{MetOO} + \text{Met} \rightarrow 2\text{MetO} \quad \Delta H_{\text{rxn}} = -244.1 \text{ kJ/mol} \quad (11)
\]

Concerning the reaction enthalpies for formation of persulfoxide and sulfoxide, the neutral system has only moderate changes compared to [Met - H]⁻. More pronounced effects are observed in their PESs and associated activation barriers. Unlike the reaction of [Met - H]⁻ + ^1\text{O}_2 (see Fig. 4a), the PES scan for Met + ^1\text{O}_2 (see Fig. 6a) results in a covalently bound persulfoxide MetOO barrierlessly, without formation of a weakly bound precursor. MetOO has the O₂ moiety sandwiched between the ammonium group and the sulfur atom. MetOO has a zwitterionic character [76], so the proton of the ammonium group is shared between the N and the terminal O, rendering MetOO an "S-hydroperoxide" compound.

When another Met approaches MetOO, the latter transfers an O atom to Met. As the O-O bond ruptures and the new O-S bond forms, two molecules of MetO are produced. The PES for this process is scanned in Fig. 6b along the distance between the terminal O of MetOO and the S of Met from 5.0 Å to 1.5 Å at a decrement of 0.05 Å each step. A transition state TS2 is located at r(O-S) of 2.37 Å and r(O-O) of 1.69 Å. At TS2, the O-O bond rupture is accompanied by the proton shuttling back to the amino group. TS2 lies 25.1 kJ/mol above the reactants (For comparison, TS2 is 13.5 kJ/mol lower than the corresponding TS2⁺ for [MetOO - H⁺] + [Met - H⁻]). The overall activation energy \( E_a \) for \( 2\text{Met} + ^1\text{O}_2 \rightarrow 2\text{MetO} \) is -57.9 kJ/mol according to eq. (9).

Based on the similarities between the PESs for \(^1\text{O}_2\) with deprotonated and neutral Met, we conclude that both systems follow similar kinetics, and have applied the pre-equilibrium assumption to eq. (10) and QSSA to MetOO. As a result, the rate law for formation of MetO can be written as
\[
\frac{1}{2} \frac{d[\text{MetO}]}{dt} = k_2[\text{MetOO}][\text{Met}] = \frac{k_f}{k_{-1}} k_2 [\text{Met}]^2 \left[ ^1 \text{O}_2 \right] = K k_2 [\text{Met}]^2 \left[ ^1 \text{O}_2 \right] = k [\text{Met}]^2 \left[ ^1 \text{O}_2 \right]
\]  

(12)

and its integrated form

\[
\frac{[\text{MetO}]}{[\text{Met}][^1 \text{O}_2]_{\text{ave}}} = 2k [\text{Met}]_0 t
\]

(13)

where \( k_f \) and \( k_{-1} \) are the rate constants for the forward and backward reactions of eq. (10), \( k_2 \) is the rate constant for eq. (11), \( K \) is the equilibrium constant for eq. (10), \( k \) is the effective rate constant for formation of MetO from 2Met + \(^1\text{O}_2\), and \([\text{Met}]_0\) is the initial Met concentration. Note that, to facilitate comparisons, all the corresponding rate constants, equilibrium constants and barriers for different ionization states have been assigned identical names, but with "+/-" superscripts to indicate ionization states. Because of the lower TS2 barrier and consequently the larger \( k_2 \) (compared to TS2- and \( k_2^- \), respectively), MetOO could react more promptly with another Met to yield MetO, and itself was therefore missing in product mass spectra. A plot of \( \frac{[\text{MetO}]}{[\text{Met}][^1 \text{O}_2]_{\text{ave}}} \) vs. reaction time shown in Fig. 5b is linear, from which the value of \( k \) is calculated to be \( 3.7 \times 10^{10} \text{ M}^{-2} \cdot \text{s}^{-1} \). Remarkably, the \( k \) for neural Met is a factor of 37 higher than the \( k^- \) for deprotonated Met.

**MetH\(^+\) presents similar oxidation behaviors as neutral Met**

To further explore the effects of ionization on the Met reaction with \(^1\text{O}_2\), we have constructed the PESs for protonated \([\text{Met} + \text{H}]^+ + \text{O}_2\) in solution, as illustrated in Fig. 6c and d. The oxidation of MetH\(^+\) follows the same reaction mechanism and shows nearly identical PES profiles and energetics as those for neutral Met, i.e., formation of S-hydroperoxide from encounter of \([\text{Met} + \text{H}]^+ + \text{O}_2\), and \([\text{Met} + \text{H}]^+\) via an activation barrier TS2\(^+\) located at 27.0 kJ/mol above reactants.

\[
\text{[Met} + \text{H}]^+ + \text{O}_2 \quad \text{[MetOO} + \text{H}]^+ \quad \Delta H_{\text{rxn}} = -96.5 \text{ kJ/mol} \quad (14)
\]

\[
\text{[MetOO} + \text{H}]^+ + \text{[Met} + \text{H}]^+ \quad 2\text{[MetO} + \text{H}]^+ \quad \Delta H_{\text{rxn}} = -235.4 \text{ kJ/mol} \quad (15)
\]

**Discussion**
Comparison with photooxidation kinetics

Matheson et al. [22] reported the reactive rate constant \( k_r (1.6 \times 10^7 \text{ M}^{-1}\text{s}^{-1}) \) and the total quenching rate constant \( k_t (1.7 \times 10^7 \text{ M}^{-1}\text{s}^{-1}) \) of \(^1\text{O}_2\) with Met, where \(^1\text{O}_2\) was generated by direct excitation of \(^3\text{O}_2\) in D\(_2\)O (pD = 8.4). \( k_t \) was evaluated by competitive inhibition of the chemical reaction of bilirubin with \(^1\text{O}_2\), and \( k_r \) was measured from loss of Met using an amino acid analyzer. Most of the other kinetic measurements for Met with \(^1\text{O}_2\) are based on dye-sensitized photooxidations. Kraljic et al. [78] reported a \( k_r \) of \( 8.6 \times 10^6 \text{ M}^{-1}\text{s}^{-1} \) in neutral water, according to O\(_2\) consumption in steady state photosensitization. Lindig et al. [79] examined \( k_t \) in neat D\(_2\)O using laser flash photolysis. Quenching of \(^1\text{O}_2\) lay in the competition for \(^1\text{O}_2\) between ADPA and Met, and the \( k_t \) for Met was measured to be \( 1.5 \times 10^7 \text{ M}^{-1}\text{s}^{-1} \). In the experiment of Miskoski and García [23], \(^1\text{O}_2\) was generated by rose bengel and tryptophan was used as a sacrificial substrate. The solution was photolyzed, and tryptophan consumption was monitored by its fluorescence in the absence and the presence of Met. Through a Stern-Volmer analysis of dynamic quenching of fluorescence, a \( k_t \) of \( 2.1 \times 10^{-7} \text{ M}^{-1}\text{s}^{-1} \) was obtained for Met at pH 7. \( k_r \) was measured based on O\(_2\) consumption in a solution containing sensitizer and Met, and its value equals \( k_t \). Based on a similar fluorescence quenching approach with diphenylfuran as a fluorescence probe, Sysak et al. reported a \( k_r \) of \( 1.15 \times 10^7 \text{ M}^{-1}\text{s}^{-1} \) in water (pH = 7) [24].

In above works, no kinetic model was given and all of the \( k_r \) values were reported as a second-order rate constant in the unit of M\(^{-1}\)s\(^{-1}\). If we assumed that formation of MetOO is the rate-limiting step for Met oxidation, the rate law for formation of MetO would follow second-order kinetics as eq. (16). Least-square fitting of our kinetics data to eq. (16) gave a \( k_i \) value of \( 1.6 \times 10^7 \text{ M}^{-1}\text{s}^{-1} \), similar as those reported from photooxidations. But our data were poorly fitted to eq. (16), suggesting eq. (16) does not describe kinetics correctly.

\[
\ln \frac{[\text{Met}]}{[\text{Met}]-[\text{MetO}]} = 2k_i[\text{O}_2]_{\text{ave}}t
\]  

(16)

The pH dependence of Met photooxidation has also been subjected to scrutiny. Earlier work on methylene blue-sensitized photooxidation of Met by Weil [80] and Spikes et al. [27] showed a remarkable
increase of O$_2$ consumption at a high pH range starting from 8. Sysak et al. [24] reported comprehensive pH dependence of Met photooxidation. According to them, the reaction is initialized by formation of a persulfoxide intermediate, followed by secondary reactions that are both pH- and buffer-dependent. At high pH (above 9) and catalyzed by buffer ions, OH$^-$ may attack the S of persulfoxide, and the reaction gives one molecule of sulfoxide and one molecule of H$_2$O$_2$. At intermediate pH of 6 - 10, when Met carries a free amino group, the dominant pathway leads to dehydromethionine and H$_2$O$_2$ via internal displacement, where the dehydromethionine was assigned as a five-membered heterocyclic N-S compound [24]. Dehydromethionine may slowly hydrolyze to Met sulfoxide. At pH below 6, the persulfoxide oxidizes a second Met by the sulfide trapping mechanism, resulting in a stoichiometry of 2Met + $^1$O$_2$ → 2MetO. Competition among the secondary reactions accounts for the variation in O$_2$ uptake at different pH, i.e., the Met to $^1$O$_2$ ratio is 0.85:1 above pH 8 and 2:1 below pH 5 [24, 65].

It is worth mentioning that formation of dehydromethionine in photooxidations was usually determined based on -NH$_2$ loss (using fluorogenic detection), but the loss of primary -NH$_2$ reactivity strongly depended on dyes [81] and buffer ions [24]. For example, little change was observed in the -NH$_2$ reactivity when eosin rather than methylene blue was used as a sensitizer [81]. It was suspected that the excited dye abstracted H atom from Met to form dehydromethionine [24, 80]. This hypothesis was corroborated by the fact that H$_2$O$_2$ was formed in methylene blue radical-mediated photooxidation of Met [80]. This implies that formation of dehydromethionine and H$_2$O$_2$ might not be completely $^1$O$_2$-specific [24], and the observed photooxidation pH dependence may reflect the oxidation-reduction potential between Met and excited dyes[80]. The uncertainty in photooxidation kinetics also remains as to whether the O$_2$ uptake is dedicated to $^1$O$_2$-induced oxidation or partially due to formation and reactions of -O$_2^-$ with substrates. Such uncertainty was eliminated in our measurements, by bubbling "clean" $^1$O$_2$ to the Met solution, and using a straightforward MS technique to identify products.

**Evolution of Met oxidation from the gas phase to solution**

Preceding the present solution work, we reported the reactions of Met ions with $^1$O$_2$ in the gas phase.
We first investigated the oxidation of protonated and deprotonated Met in the absence of water [35, 36]. The reaction systems were then augmented by adding explicit number of water ligands [35, 36], which accounts for microsolvation. The parallel gas- and solution-phase study helps us evaluate evolution of Met oxidation dynamics and kinetics from the gas phase, through microsolvation, to the aqueous solution.

The gas-phase oxidation processes follow an addition/elimination mechanism. The first step is addition of the O$_2$ moiety to the Met sulfur, yielding persulfoxide. The reaction outcome is determined by whether the persulfoxide intermediate has accessible product channels or not. In the case of dehydrated [Met $+$ H$^+$] + O$_2$, the persulfoxide intermediate evolves to an S-hydroperoxide HN$_2$CH(CO$_2$H)CH$_2$CH$_2$S(OOH$^+$)CH$_3$. The latter eliminates H$_2$O$_2$, yielding a dehydro compound of MetH$^+$. The reaction is extremely efficient at low $E_{\text{col}}$, approaching the collision limit. In the reaction of dehydrated [Met - H$^-$], the persulfoxide intermediate interconverts to S-hydroperoxides NH$_2$CH(CO$_2^-$)CH$_2$CH$_2$S(OOH$^>$)CH$_2$CH$_2$S(OOH$^>$)CH$_3$. Contrary to the protonated case, H$_2$O$_2$ elimination of deprotonated S-hydroperoxides encounters insurmountable barriers. Therefore, none of deprotonated S-hydroperoxides could convert to stable end-products but decayed back to reactants, making [Met - H$^-$] non-reactive towards O$_2$.

The fates of S-hydroperoxide intermediates are changed once [Met $+$ H$^+$] and [Met - H$^-$] become hydrated. The dissociation of water ligand(s) provides an energy disposal path to release the heat gained from peroxide formation. It enables the capture of the "relaxed" S-hydroperoxides as stable end-products, i.e., HN$_2$CH(CO$_2$H)CH$_2$CH$_2$S(OOH$^+$)CH$_3$ for oxidation of [Met $+$ H$^+$](H$_2$O)$_{1,2}$, and NH$_2$CH(CO$_2^-$)CH$_2$CH$_2$S(OOH$^>$)CH$_2$CH$_2$S(OOH$^>$)CH$_3$ for [Met - H$^-$](H$_2$O)$_{1,2}$. And for the reactions of [Met $+$ H$^+$](H$_2$O)$_{1,2}$, H$_2$O$_2$ elimination becomes less dominant.

Interestingly, reaction efficiencies of [Met $+$ H$^+$](H$_2$O) and [Met + H$^+$](H$_2$O)$_2$ are 15 and 25 times higher than those of [Met - H$^-$](H$_2$O) and [Met - H$^-$](H$_2$O)$_2$, respectively. The effect of ionization state on gas-phase reaction efficiencies can be traced back to the formation efficiencies of protonated and deprotonated S-hydroperoxides, whose structures are completely different. Formation of protonated S-
hydroperoxide $\text{HN}_{2}\text{CH(CO}_2\text{H})\text{CH}_2\text{CH}_2\text{S(OOH}^+)\text{CH}_3$ takes place via intramolecular proton transfer from the $[\text{Met} + \text{H}]^+$ ammonium group to -SOO, and there is no activation barrier. On the other hand, deprotonated S-hydroperoxide $\text{NH}_2\text{CH(CO}_2\text{)}\text{CH}_2\text{CH}_2\text{S(OOH}=\text{CH}_2$ or $\text{NH}_2\text{CH(CO}_2\text{)}\text{CH}_2\text{CH}=\text{S(OOH)}\text{CH}_3$ is formed by taking an H from the terminal -SCH$_3$ or from the $\gamma$-CH$_2$; neither of these two routes is facile.

Similar to gas-phase reactions, the first step of Met oxidation in solution is formation of persulfoxide. However, only in acidic solution the initially formed persulfoxide evolves to S-hydroperoxide; in basic solution, the structure of initial persulfoxide remains stable presumably due to bulk solvent stabilization. In aqueous solution, persulfoxide or S-hydroperoxide could be thermalized through solute-solvent interactions. However, a downstream route opens for persulfoxide/S-hydroperoxide through the participation of other Met molecules, leading to sulfoxide.

Different than the gas-phase reactions where the reaction efficiencies mostly depend on the formation efficiencies of S-hydroperoxides, the reaction efficiencies in solution are also related to the second step which leads to formation of sulfoxides by O-transfer between initially formed persulfoxide and second Met. Based on DFT-PCM calculations, the barriers for this reaction are 27.0, 25.1 and 38.6 kJ/mol for protonated, neutral and deprotonated systems, respectively.

**Conclusions**

A new on-line reaction setup was developed to couple solution-phase reactions of electronically excited $^1\text{O}_2$ with an ESI mass spectrometer and an absorption spectrometer. This apparatus enables us to measure $^1\text{O}_2$ oxidation kinetics in aqueous solution. To determine $[^1\text{O}_2]$ in solution on an absolute scale, a method was established to correlate the $[^1\text{O}_2]$ in aqueous solution with airborne $^1\text{O}_2$ emission intensity. This on-line monitoring system was utilized to study the reaction kinetics of Met with $^1\text{O}_2$ at different pH. In both acidic and basic solutions, sulfoxide $[\text{MetO} - \text{H}]^-$ and $[\text{MetO} + \text{H}]^+$ were confirmed to be major products. We have captured the persulfoxide intermediates in basic solution. The reaction mechanism and pH dependence were elucidated with the assistance from DFT calculations, using the polarized
continuum model to simulate bulk solvation effects. It was found that the reactions in acidic and basic media follow the same mechanism composed of two elementary steps, formation of persulfoxide for deprotonated Met or S-hydroperoxide for neutral/protonated Met, followed by transfer of an O atom from persulfoxide or S-hydroperoxide to a second Met, producing two sulfoxides. The second step is rate-limiting, and formation of sulfoxide follows third-order rate law with respect to [Met]^2[1O2]. A remarkable result concerns the pH dependence of Met oxidation. The effective rate constants for formation of Met sulfoxide are $3.7 \times 10^{10} \text{ M}^{-2}\cdot\text{s}^{-1}$ at pH 3.2, decreasing to $1.0 \times 10^{9} \text{ M}^{-2}\cdot\text{s}^{-1}$ at pH 10.4. The significantly lower rate constant in basic solution is due to the high activation barrier leading to formation of sulfoxide from deprotonated persulfoxide, and the latter overwhelmingly decayed back to reactants accompanied by physical quenching of $^1\text{O}_2$.

Combined with our findings in the gas-phase $^1\text{O}_2$ oxidation of hydrated/dehydrated Met ions, a panorama can be created for evolution of the Met oxidation mechanism from the gas phase, through microsolvation, to aqueous solution. Reactions in different media and at different ionization states are all mediated by persulfoxides, which carry high internal energy and thus are very reactive. In the gas phase, the fates of persulfoxides are determined by whether they have exit product channels or not. In the reaction of protonated Met, transfer of two H from [Met + H]^+ to O$_2$ provides an energetically favored dissociation path. To the contrary, H$_2$O$_2$ elimination is inhibited in deprotonated persulfoxide, and the latter could only decay back to reactants. The fates of persulfoxide intermediates are altered upon microsolvation in the gas phase. Dominant hydration effect is the suppression of persulfoxide decomposition. This can be attributed to energy dissipation from excited persulfoxide intermediates via water dissociation, yielding persulfoxides themselves as stable products. Finally, in solution-phase reactions, persulfoxide intermediates, once formed, continue to react with another molecule of Met, leading to formation of sulfoxides as final products.
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Electronic supplementary material: UV-Vis absorption spectra showing $^{1}\text{O}_2$-induced oxidation of uric acid.

References


Figure captions

Fig. 1 Schematic depiction of on-line coupling of $^1$O$_2$ generation, detection and reaction to spectroscopy and ESI MS. The configuration of ESI is shown for negative ion mode. For positive ESI, aqueous solution from sampling loop is directly transferred to an ESI needle through PEEK tubing. 1) Sparger; 2) cold trap; 3) optical emission cell; 4) InGaAs detector; 5) reaction vessel; 6) pressure relay; 7) piston pump; 8) peristaltic pump; 9) quartz flow cell; 10) diode array spectrometer; 11) 2-position switching valve; 12a/b) syringe pump; 13) borosilicate theta-glass capillary; 14a/b) PEEK tubing; 15) PEEK microtee; and 16) platinum wire.

Fig. 2 (a) UV-Vis absorption spectra of ADPA over the course of the reaction with $^1$O$_2$, recorded at pH 10. The insert shows the plot of $\ln(A_t/A_0)$ vs. reaction time, where $A_t$ and $A_0$ are the absorbance at 378 nm at different times and time zero, respectively; and (b) The linear relationship between the emission intensity of airborne $^1$O$_2$ and the [1O$_2$] in solution determined using ADPA trapping.

Fig. 3 (a) Product mass spectra for Met oxidation in pH 10.4 solution at different reaction times; and (b) Plot of $\frac{[\text{MetO} - \text{H}]}{[\text{Met} - \text{H}][1O_2]_{ave}}$ vs. reaction time $t$.

Fig. 4 (a) PES for the reaction of [Met - H] + $^1$O$_2$ → [MetOO - H], plotted against the distance between the S atom of [Met - H] and the closer O atom of O$_2$; and (b) 2D PES for the reaction of [MetOO - H] + [Met - H] → 2[MetO - H], plotted against the O-O bond length and the distance between the terminal O of [MetOO - H] and the S of [Met - H]. Calculations were carried out at B3LYP/PCM/6-31+G*.

Fig. 5 (a) Product mass spectra for Met oxidation in pH 3.2 solution at different reaction times; and (b) Plot of $\frac{[\text{MetO}]}{[\text{Met}][1O_2]_{ave}}$ vs. reaction time $t$.

Fig. 6 (a) PES for the reaction of Met + $^1$O$_2$ → MetOO, plotted against the distance between the Met S atom and the closer O atom of O$_2$; (b) PES for the reaction of MetOO + Met → 2MetO, plotted against the distance between the terminal O of MetOO and the S of Met; (c) and (d) are similar to (a) and (b), except that the Met, persulfoxide and sulfoxide are protonated in (c) and (d). Calculations were carried out at B3LYP/PCM/6-31+G*.
Fig. 2

(a) Absorbance vs. Wavelength (nm) with inset showing ln(A/A₀) vs. Time (x 10 s).

(b) [¹O₂] in solution (x 10⁻¹² M) vs. ¹O₂ emission intensity (mV).
Fig. 3

**a)**

- **Primary Ion**
  - [Met - H]
  - m/z 148

- **Product Ion 1**
  - [MetO - H] (x 4)
  - m/z 184

- **Product Ion 2**
  - [MetOO - H] (x 10)
  - m/z 189

- **Product Ion 3**
  - [MetO - H - Met]
  - m/z 313 (x 10)

**b)**

Graph showing the relationship between reaction time (sec) and the sum of products over reactant times [O₂]ave.
Fig. 5

(a) Mass spectra showing the primary ion 
[Met + H]^+ at m/z 150 and the product ion 
([Met + H]^+ - O) at m/z 166.

(b) Graph showing the product (Product / (Reactant x [O2]ave)) vs. reaction time (sec) with a linear trend.

Ion Intensity (counts/s)

Product / (Reactant x [O2]ave)

Reaction Time (sec)
Fig. 6

Potential Energy (kJ/mol) vs. r(O-S) (Å)

(a) Met + ^1O_2
(b) MetOO + Met
(c) [Met + H]^+ + ^1O_2
(d) TS2

Potential Energy (kJ/mol) vs. r(O-S) (Å)

[TS2] converts to S-hydroperoxide
[2 x MetO]