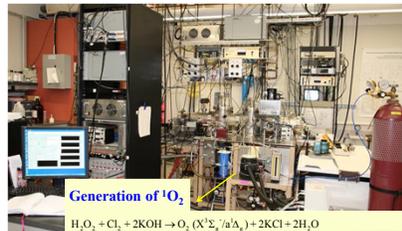


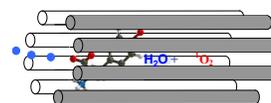
¹O₂-Induced Oxidation of Methionine (Met) in Biological Systems

- ❖ Oxidation of Met residues produces MetO. This process protects other buried residues under oxidative stress.
- ❖ Cells develop a counteract process to reduce MetO back to Met, catalyzed by methionine sulfoxide reductases (Msr). Consequently, the content of Met and MetO in the cells can be used to indicate the increase of reactive oxygen species generation, the decrease of oxidant scavengers and/or Msrs, and as a marker for biological aging.
- ❖ It is recently established that Met residues form hydrophobic bonds between their S atoms and the aromatic rings of other residues. The sulfur-ring bonds stabilize the protein structure. However, MetO is hydrophilic, and the normal 3-dimensional folding of proteins will dissociate under alteration from Met to MetO. This may cause proteins to lose function, and is related to pathophysiological conditions such as cancer, aging, and neurodegenerative diseases.

ESI Guided-Ion-Beam Tandem Mass Spectrometer



Ion-Molecule Scattering of Amino Acid Ions with ¹O₂

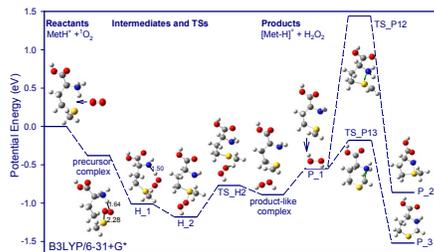
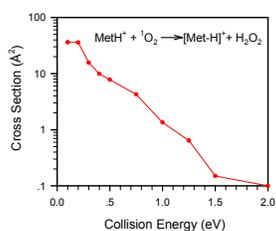


Computational Modeling

- ❖ DFT calculations and RRKM modeling were used to examine properties of complexes, transition states, and products along reaction coordinates.
- ❖ Quasi-classical, direct dynamics trajectory simulations were carried out at the B3LYP/4-31G* level of theory, using Hase's Venus to set up initial conditions and Gaussian 09 to integrate trajectories.

I. Oxidation of isolated Met in the gas phase, using guided-ion-beam scattering methods

MetH⁺ + ¹O₂

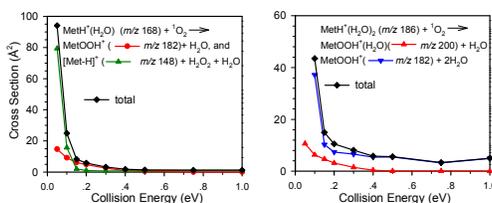


Y. Fang, F. Liu, A. Bennett, S. Ara, and J. Liu, *J. Phys. Chem. B*, 2011, 115, 2671-2682.

[Met - H]⁻ + ¹O₂

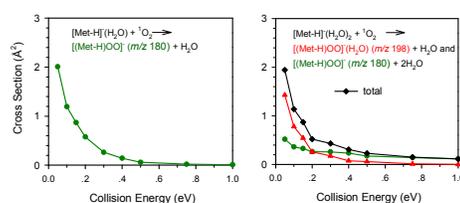
No oxidation products were observed

MetH⁺(H₂O)_{1,2} + ¹O₂

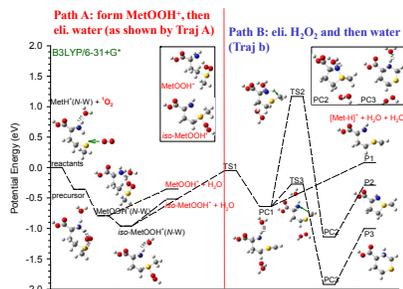


- ❖ Oxidation of MetH⁺(H₂O) leads to hydroperoxide MetOOH⁺, which may further undergo H₂O₂ elimination.
- ❖ Addition of the second water to MetH⁺(H₂O) shuts down the H₂O₂ elimination channel, yielding MetOOH⁺(H₂O)_n only.
- ❖ In contrast to non-reactivity of [Met - H]⁻, [Met - H]⁻(H₂O)_n are vulnerable to ¹O₂ and produces [Met - H]⁻-OO, albeit with lower reactivity compared to MetH⁺(H₂O)_n.
- ❖ Most importantly, we were able to capture the peroxide intermediates in the gas-phase hydrated clusters.

[Met - H]⁻(H₂O)_{1,2} + ¹O₂

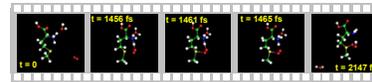


Potential energy surface for the reaction of MetH⁺(H₂O) with ¹O₂

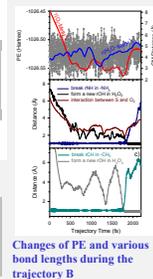
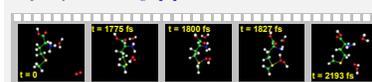


Direct-Dynamics Trajectories for MetH⁺(H₂O) + ¹O₂ at E_{col} = 0.1 eV

Trajectory A: forming MetOOH⁺, followed by water elimination

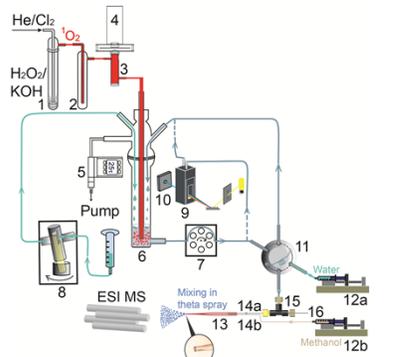


Trajectory B: eliminating H₂O₂ and then water



III. Oxidation of Met in solution: Fates of peroxides in solution

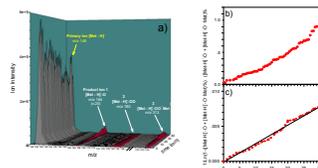
On-line spectroscopy and MS monitoring of solution-phase ¹O₂ reactions



- 1) sparger, 2) cold trap, 3) emission cell, 4) InGaAs detector, 5) pressure relay, 6) reaction vessel, 7) peristaltic pump, 8) rotary piston pump, 9) quartz flow cell, 10) UV-Vis diode array spectrometer, 11) 2-position switching valve, 12a) syringe pumps, 13) theta ESI capillary, 14a) aqueous solution, 14b) methanol makeup, 15) PEEK micro-tec, 16) platinum wire for ESI voltage

Products and kinetics in basic solution

1. $[Met - H]^{-} (m/z 148) + {}^1O_2 \xrightarrow{k_1^-} [Met - H]^{-}OO (m/z 180)$, rate-limiting step, $k_1^- = 2.1 \times 10^6 M^{-1} \cdot s^{-1}$
2. $[Met - H]^{-}OO + [Met - H]^{-} \xrightarrow{k_2^-} 2[Met - H]^{-}O (m/z 164)$, and $\frac{d([Met - H]^{-}OO)}{dt} = 0$
3. $[Met - H]^{-}O + Met \xrightarrow{k_3^-} [Met - H]^{-}O \cdots Met (m/z 313)$, H-bonded dimer, $k_3^- = 1.5 M^{-1} \cdot s^{-1}$

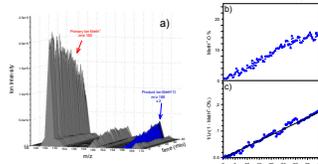


(a) Time profiles and (b, c) kinetics analysis of [Met - H]⁻-O and [Met - H]⁻-O-Met.

Reaction intermediate [Met - H]⁻-OO was observed in solution, and can be treated with pseudo-steady-state approximation.

Products and kinetics in acidic solution

1. $MetH^{+} (m/z 150) + {}^1O_2 \xrightarrow{k_1^+} MetH^{+}OO$, rate-limiting step, $k_1^+ = 1.9 \times 10^7 M^{-1} \cdot s^{-1}$
2. $MetH^{+}OO + Met \xrightarrow{k_2^+} 2MetH^{+}O (m/z 166)$, and $\frac{d(MetH^{+}OO)}{dt} = 0$



(a) Time profiles and (b, c) kinetics analysis of the product MetH⁺-O.

Reaction intermediate MetH⁺-OO is more reactive than its negative counterpart, and we were not able to detect it in solution.

Conclusions

- ❖ The oxidation of Met is mediated by a hydroperoxide.
- ❖ The hydroperoxide dissociates in the gas phase by H₂O₂ elimination.
- ❖ However, the hydroperoxide can be stabilized in gas-phase hydrated clusters by water evaporation.
- ❖ In aqueous solution, hydroperoxides further react with another molecule of Met, leading to ultimate products MetO.
- ❖ Oxidation of Met in all different media present similar pH dependence, with the protonated form being more reactive than the deprotonated ones.
- ❖ Gas-phase hydrated clusters provide a promising platform to elucidate reaction intermediates, and bridge the gas-phase and solution chemistry.

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