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Redox Photochemistry of Methionine by Sulfur K-edge X-ray Absorption Spectroscopy: Potential Implications for Cataract Formation

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Abstract: The photochemistry of methionine, methionine sulfoxide, and methionine sulfone have been investigated by using sulfur K-edge X-ray absorption spectroscopy to explore the redox photochemical processes under different conditions. Methionine is easily photooxidized to the sulfoxide and the sulfone in the presence of dioxygen. In the absence of oxidant, photoirradiation leads to the one-electron-oxidized cation radical with no further reaction, suggesting that an alternative mechanism for photooxidation of thioethers through direct oxidation is feasible. The photochemistry of methionine sulfoxide allows for *independent* oxidative and reductive processes. Photoreduction of the sulfoxide leads back to the parent thioether under both aerobic and anaerobic conditions. Photooxidation occurs only under aerobic conditions. In contrast, methionine sulfone is photochemically inert. These results provide new insights into potential photochemical processes that may lead to cataract formation.

Introduction

Oxidative damage to proteins has been extensively studied in relation to disease states such as diabetes, atherosclerosis, and neurodegenerative diseases.[1] Among the natural amino acids, methionine is remarkable for its high susceptibility to oxidation.[2] Formal oxidation of methionine (MetS) is generally considered to involve two oxo transfer steps, producing methionine sulfoxide (MetSO) in a facile first oxidation and methionine sulfone ($MetSO_2$) under harsher conditions (Scheme 1). MetSO has been documented in native proteins, indicating that the first oxidation of the methionine side chain is a physiologically relevant phenomenon.[3] The chemical effects of MetSO formation include (*i*) an increase in the steric bulk and a decrease in the flexibility of the side chain,[4] (*ii*) a significant increase in the polarity/hydrophilicity of the side chain,[5] (*iii*) the formation of a new H-bond acceptor site,[6] and (*iv*) shutdown of its function as a methyl donor.[7,8]

Organisms have evolved complex antioxidant defense mechanisms to minimize oxidative damage to proteins and other macromolecules. They also possess repair systems for reversing some oxidative modifications. While oxidation to $MetSO_2$ is considered to be biologically irreversible, MetSO can be physiologically reduced back to MetS.[9] Reduction of MetSO to MetS is catalyzed by methionine sulfoxide reductases (Msr's) in the presence of thioredoxin as the in vivo sacrificial reductant. Two classes of Msr enzymes are known, depending on the stereochemistry at the sulfur center: MsrA reduces (*S*)-MetSO, whereas MsrB preferentially reduces (*R*)-MetSO.[10,11] Msr's are relatively small cytosolic enzymes found in a variety of organisms ranging from bacteria to plants and animals, including humans;[12−15] they can reduce both the free MetSO amino acid and MetSO within peptides and proteins.[10,16] The capability of organisms to reverse oxidative damage to MetS side chains suggests that MetS oxidation may serve to regulate protein activity.[17,18] However, poor regulation of this oxidation/reduction of MetS can lead to disease states.

There is a considerable amount of data indicating that the brain in Alzheimer's disease is under increased oxidative stress[19] and that this plays a likely role in the pathogenesis of neuronal

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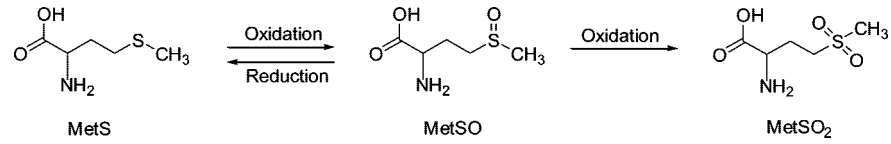
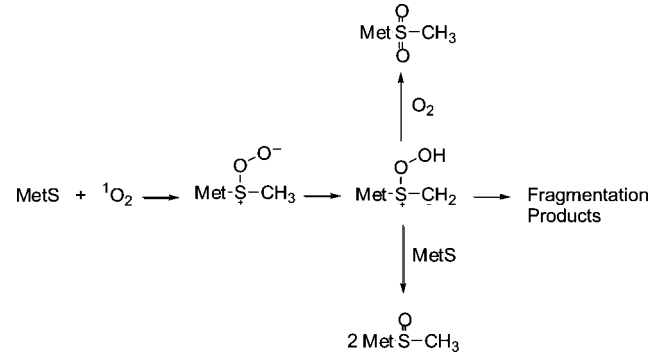
Scheme 1

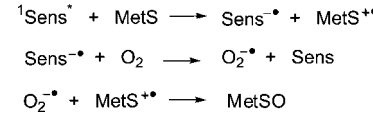


degeneration and ultimately death in Alzheimer's patients.[20] It has been postulated that MetS oxidation might be crucial for both the aggregation and neurotoxicity of amyloid β peptide, a major component in the core of senile plaques associated with Alzheimer's disease.[21] Oxidative damage is also thought to be a major factor in the development of age-related cataracts, a leading cause of blindness in the world.[22] Age-related cataracts are characterized by extensive oxidation, cross-linking, and insolubilization of lens proteins.[23] One of the most abundant lens proteins, α-crystallin, is found extensively in cataract protein aggregates. Its structural function is to assist in maintaining the proper refractive index in the lens.[24] In addition, it has been shown to function as a molecular chaperone, helping to prevent formation of large light-scattering aggregates.[25] Oxidation of α-crystallin has been demonstrated to cause a significant reduction in its chaperone activity, which suggests that oxidation may have important consequences for protein aggregation in the lens.[26] Available data indicates that photooxidation is involved in the oxidation of α-crystallin. MetS oxidation in both bovine and human α-crystallin occurs in vivo,[27,28] and long-term UV light exposure increases oxidation of α-crystallin, specifically methionine and cysteine residues.[29,30] In general, very little UV-C (<280 nm) radiation reaches the earth's surface because of atmospheric absorption in the ozone layer. However, UV-B (280−320 nm) and UV-A (320−400 nm) can reach the eye, and the human cornea filters out all radiation below 290 nm. UV-B radiation is generally absorbed by the cornea and aqueous humor before reaching the lens,[31] yet UV-B exposure over extended periods has been shown to cause damage to the lens that can lead to cataract formation.[32−34] It has also been proposed that higher-wavelength UV-A can also cause damage to the lens, again leading to cataract formation.[35,36] However, the mechanism of light-induced cataract formation is still not clear, although recent studies suggest that dioxygen permeability into the crystalline lens likely plays an important role.[37]

Scheme 2



Scheme 3



Much effort has been devoted to studying the mechanism of *sensitized* photooxidation of functionalized thioethers.[38,39] Under such conditions, the mechanism of photooxidation is believed to involve the formation of $^1O_2$, as shown in Scheme 2. Foote and Peters[40] and Foote and co-workers[41] showed that $^1O_2$ reacts with dialkyl sulfides to form a persulfoxide intermediate, which can react with another thioether to form the sulfoxide. An alternative mechanism involves photosensitized electron transfer (ET) oxidation (Scheme 3), where the sulfide radical cation forms upon ET to the excited sensitizer, which is then regenerated by reaction with oxygen to yield the superoxide anion.[42] Recombination of the sulfide radical cation and the superoxide anion yields the sulfoxide. It has not been demonstrated, however, that the photochemistry of methionine and its related oxidized species behaves similarly *in the absence of a photosensitizer*.

In this study, we explore the photochemistry of MetS, MetSO, and $MetSO_2$ using sulfur K-edge X-ray absorption spectroscopy (XAS). The element specificity of XAS enables direct evaluation

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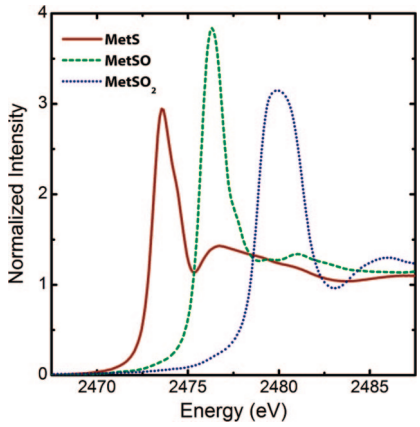

Figure 1. S K-edge XAS spectra of MetS, MetSO, and $MetSO_2$ as labeled in the accompanying legend. The intense main feature observed in each of the S K-edge spectra is very sensitive to the oxidation state of the sulfur atom.

of redox processes occurring at the atom of interest. Figure 1 shows the S K-edge XAS spectra of MetS, MetSO, and $MetSO_2$, which clearly indicate the effect of sulfur oxidation. The clearly resolved intense pre-edge feature observed for MetS (2473.5 eV), MetSO (2476.3 eV), and $MetSO_2$ (2479.9 eV) increases in energy as a function of oxidation state, primarily as a result of a decrease in the energy of the S 1s donor orbital with greater oxidation.[43,44] Our work demonstrates that the photochemistry of MetS, MetSO, and $MetSO_2$ is significantly richer than previously recognized, potentially leading to new insights into factors that can affect photochemically activated amino acid redox processes.

Materials and Methods

Materials. L-Methionine (purity 98.0%) and L-methionine sulfoxide (purity 99.0%) were used as purchased from Sigma-Aldrich. L-Methionine sulfone (purity 99.0%) was used as purchased from MP Biomedicals. Sulfur-free Kapton tape was purchased from Creative Global Services and checked for sulfur contamination using sulfur K-edge XAS before use.

Sample Preparation. Solid samples were finely ground, placed on Kapton tape, and mounted across the window of an Al plate. Samples were exposed to irradiation from a 75 W Xe arc lamp for 0−3 h at room temperature followed by analysis using XAS. Anaerobic photochemical experiments were performed in a He glovebag, and XAS data were collected in situ (see below). Solution samples of Met or MetSO were prepared at a concentration of 10 mM in distilled water with 50% glycerol as a glassing agent to reduce diffraction produced by ice crystals in XAS. For ^1H NMR analysis, ground samples were irradiated on a clean Petri dish and then dissolved in D_2O for spectroscopic analysis. ^1H NMR spectra were collected on a Bruker Avance 400 MHz spectrometer at ambient temperature. All of the photochemistry was performed in the solid state or in 10 mM aqueous solutions, with qualitatively identical results. Only the solid-state data are reported and discussed herein.

XAS Data Collection. XAS data were collected at beamline S06-2 of the Stanford Synchrotron Radiation Laboratory (SSRL) under ring conditions of 3 GeV and 60−100 mA. The setup used a 54-pole wiggler beamline operating in high-field (10 kG) mode with a Ni-coated harmonic rejection mirror and a fully tuned Si(III) double-crystal monochromator. Energy calibrations were carried out using an external standard of sodium thiosulfate ($Na_2S_2O_3$) with the first pre-edge feature being calibrated at 2472.02 eV. Signals were detected with a N_2 fluorescence (Lytle) detector at the temperature of liquid He. Details of the in situ irradiation setup are described elsewhere.[45]

Data Processing and Analysis. SIXPack software was used for XAS data reduction.[46] Pre- and postdata calibration scans were compared, and the energy scale of the data was adjusted by taking the first derivative of the first pre-edge feature of $Na_2S_2O_3$ and setting this to 2472.02 eV. Where appropriate, data scans were averaged and a smooth second-order polynomial background was fit to the pre-edge region and subtracted from the entire spectrum. Normalization of the data was accomplished by fitting a flattened second-order polynomial to the post-edge region and normalizing to an edge jump of 1.0.

SIXPack software was also used for principal component analysis (PCA), target transformation, and linear least-squares fitting. PCA was used to determine the smallest number of principal components (PCs) required to sufficiently describe the composite data set. During target transformation, SPOIL value ranges as defined by Malinowski[46b] were interpreted as follows: acceptable (<3), moderately acceptable (3−6), and unacceptable (>6). Linear least-squares fitting of reference spectra was used to evaluate component spectra over the energy range from 2465 to 2490 eV.

Results

MetS Photochemistry. Photoirradiation of MetS under aerobic conditions produces rapid photodegradation of the thioether, as observed by the large changes in the S K-edge XAS spectra as a function of irradiation time (Figure 2A). The intense main feature of the MetS XAS spectrum at ~2473.5 eV corresponds to the thioether CS $\sigma^* \leftarrow$ S 1s transition.[47] Upon irradiation, this feature decays with the formation of new, intense pre-edge features at 2476.3 eV and 2479.9 eV, corresponding to the formation of MetSO and $MetSO_2$, respectively. At longer irradiation times, further changes are observed, including the appearance of higher-energy features at 2481.0 and 2482.5 eV that presumably are due to fragmentation and formation of highly oxidized species. The feature at 2481.0 eV is consistent with the formation of a sulfonate species, given its similarity to the pre-edge feature of methane sulfonate ($CH_3SO_3^-$) (see S1 in the Supporting Information). The species corresponding to the feature at 2482.5 eV has not yet been identified.

Under anaerobic conditions, MetS photoirradiation does not produce MetSO or $MetSO_2$ (Figure 2B), presumably because of the lack of an oxygen source. However, careful investigation of the time-resolved XAS spectra indicates the formation of a small pre-edge feature at low energy (~2470.0 eV; see Figure 2C) concomitant with a small decrease in the CS $\sigma^* \leftarrow$ S 1s feature of MetS, which eventually achieve an apparent photosteady state (Figure 2D). We have previously shown that such low-energy pre-edge features are observed for radical species centered on the sulfur atom.[48] We therefore assign this feature to the formation of the one-electron-photooxidized species

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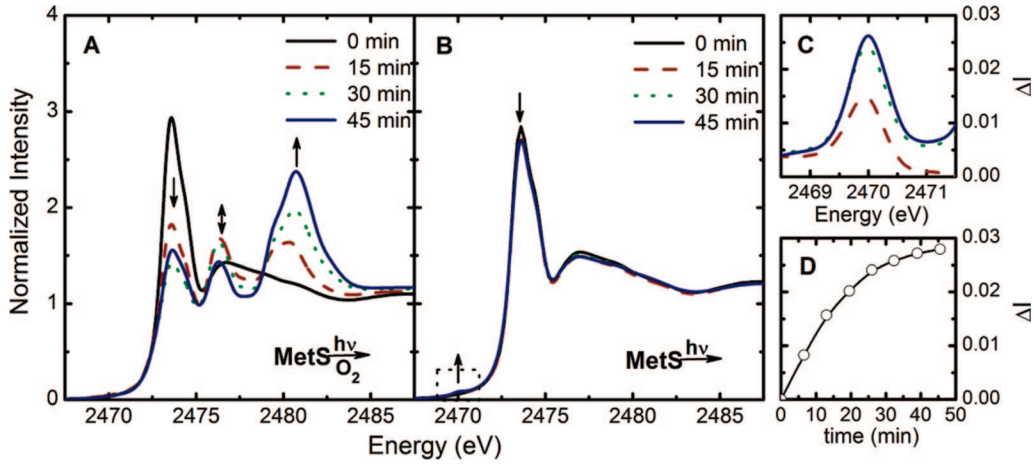
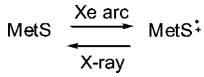


Figure 2. Photochemistry of MetS under (A) aerobic and (B) anaerobic conditions. (C) Difference spectra in the low-energy region of the spectra under anaerobic conditions, showing the behavior of a weak feature at ~2470 eV. (D) Kinetic profile of the weak pre-edge feature shown in (C), attributed to the formation of the methionine cation radical (see the text).

Scheme 4



$MetS^{\bullet+}$, which has been observed previously under different oxidation conditions.[49] Formation of a photosteady state during XAS data acquisition can easily result from concomitant X-ray photoreduction of the cation radical to regenerate MetS, as shown in Scheme 4. Decarboxylation of the cation radical has been observed at room temperature[50,51] but was not observed in our experiments at ~20 K.

MetSO Photochemistry. The results of MetSO photoirradiation under aerobic conditions are shown in Figure 3A. As with MetS photoirradiation, formation of $MetSO_2$ is observed at relatively short times, whereas more highly oxidized species, likely due to fragmentation products, are observed at very long times. However, MetSO photoirradiation also results in the formation of a new feature at lower energy, indicative of photoreductive processes. An intense feature at 2473.5 eV, corresponding to the spectrum of the parent thioether MetS, is clearly observed during photoirradiation. Photoreduction is also observed as a result of photodamage from the probe X-ray beam during data acquisition, but the rate of formation is markedly smaller than that observed with UV−vis photolysis (see S2 in the Supporting Information). The photolysis does not result from simple acceleration of the X-ray photoreduction, since ex situ photolysis (not shown) yields the same result as reported herein.

In the absence of O_2, MetSO photoirradiation shows no evidence of oxidation products (Figure 3B), but the lower-energy feature at 2473.5 eV is still observed as the MetSO features simultaneously decrease in intensity. MetSO photoreduction was confirmed by ^{1}H NMR analysis (Figure 3C), which indicated the formation of MetS after photoirradiation (both in the presence and in the absence of O_2).[41]

A more quantitative analysis of the photoreductive process was obtained through PCA and target transformation to identify the number and types of components, respectively. The first two PCA components of the XAS spectra of irradiated MetSO accounted for over 99% of the total variance (see Table 1). The first two components, with a total variance of 99.4%, reproduce all of the features of the spectra, confirming that anaerobic irradiation of MetSO yields only one product at short irradiation times. On the basis of these results, target transformation was performed using two components in order to identify the two species. According to the calculated SPOIL values (S4 in the Supporting Information) and visual inspection of the target transforms, the species were identified as MetSO and MetS. Linear least-squares fitting of the two components yielded a reasonable fit to the data (see Figure 3D).

$MetSO_2$ Photochemistry. $MetSO_2$ is both oxidatively and reductively inert under our experimental photolysis conditions. A small background reaction was observed and identified as resulting from MetSO contamination undergoing photoreduction (see above), as confirmed by ^{1}H NMR analysis (see S5 in the Supporting Information).[41]

Discussion

Methionine oxidation is implicated in various age-related diseases; photochemical redox processes at methionine are particularly relevant in cataracts, whose formation is correlated with long-term UV irradiation of the crystalline lens.[29] Increased protein oxidation and cross-linking are characteristics of cataract formation.[30] Physiologically irreversible oxidation of MetS residues to $MetSO_2$ in proteins should lead in many cases to the loss of function and therefore to pathophysiological situations. Evidence of $MetSO_2$ has been reported in senile cataracts and amyloid plaques in Alzheimer's disease,[52,53] and methionine oxidation to MetSO in combination with an insufficient reduction capacity should have similar consequences. Therefore, MetS oxidation can alter biochemical as well as physical properties of proteins that should be of physiological or pathophysiological relevance. Although significant literature exists on the photoredox properties of MetS in the presence of sensitizers (to enhance $^1O_2$ production), similar studies without the use of sensitizers

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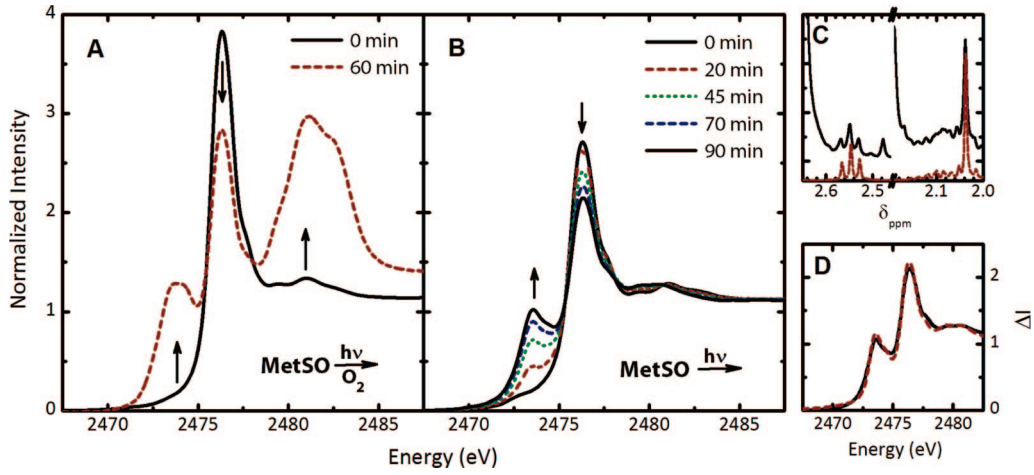

Figure 3. Photochemistry of MetS under (A) aerobic and (B) anaerobic conditions. (C) Room-temperature 400 MHz ^{1}H NMR spectrum confirming the formation of MetS during MetSO photoirradiation under anaerobic conditions after 15 min (solid black line; the red dashed line is the reference spectrum). (D) Spectrum of photoirradiated MetSO after 90 min (blue) with linear least-squares fit (red) after PCA and target transformation.

Table 1. Principal Component Analysis

component	eigenvalue	cumulative variance
1	52.378	0.924
2	3.975	0.994
3	0.159	0.997
4	0.050	0.998
5	0.025	0.998
6	0.014	0.999
7	0.012	0.999
8	0.010	0.999
9	0.009	0.999
10	0.008	1.0

have not been performed. Herein, we report novel MetS and MetSO photochemistry in the absence of photosensitizers (and in the absence of O_2), conditions of relevance to the crystalline lens.[54,55]

The mechanism of aerobic photooxidation of thioethers is generally believed to involve photoexcitation of dioxygen to its highly energetic singlet excited state followed by reaction with the thioether. Indirect photooxidation of the thioether (via an excited state of a photosensitizer) to generate a reactive thioether cation radical (Scheme 3) has also been proposed and cannot be discounted as either an alternative or concurrent pathway. Our current work suggests that a third option must be considered. *Direct* one-electron photoexcitation of MetS to form the cation radical is supported by the observation of a sulfur-centered radical during anaerobic photoirradiation of MetS *in the absence of an exogenous photosensitizer*. This is somewhat surprising given the small absorption coefficient for MetS in the visible region (see S6 in the Supporting Information). We note, however, that high-resolution electronic spectroscopy reveals weak low-lying absorption bands at 262 and 285 nm assigned as singlet-to-triplet transitions.[56,57] We postulate that these triplet excited states are sufficiently high in energy (~4 eV) to make them efficient reductants, thus yielding the cation radical. Reactivity through this triplet-excited-state channel may

Scheme 5

also point to an additional pathway for O_2 photoreactivity, as the formation of a ^{3}MetS* species should allow for a rapid spin-allowed reaction with ground-state $^3O_2$. These two pathways that may result in MetS oxidation have not been explored and provide potential alternative mechanisms for photochemically induced oxidative damage.

With respect to the impact of these photochemical processes in the formation of ocular cataracts, the human eye is potentially vulnerable to solar-derived photodamage in the 300−400 nm region, although there are several mechanisms that protect the eye from such damage.[58] As one ages, however, the effectiveness of these processes diminishes, which may lead to susceptibility in this photon energy range. The maximum absorption of the lowest-energy singlet-to-triplet transition in MetS (285 nm) extends into the 300−400 nm range, albeit with low molar absorptivity.[57] We suggest, however, that even a small amount of spectral overlap may be sufficient to cause photodamage over long time frames. We are currently moving to evaluate these ideas in greater detail.

The observed photochemistry of MetSO is particularly noteworthy, as it reveals facile oxidation to $MetSO_2$ as well as reduction to MetS in the presence of dioxygen. Harvey and Lang[59] have reported a similar photoreduction of DMSO to DMS, postulating disproportionation as the likely source of DMS. Our results are inconsistent with this interpretation, as we observe no formation of $MetSO_2$ under anaerobic conditions, whereas MetS is still produced. We have explored the photochemistry of DMSO under anaerobic conditions and observed reactivity similar to that observed for MetSO (see S7 in the Supporting Information). We are presently investigating the mechanism of this process to elucidate the details of this reaction.

Lastly, we note that the sulfone product $MetSO_2$ is a photochemical dead-end, with little photoreduction or photooxidation (S5 in the Supporting Information). There is some

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evidence that photofragmentation does occur (as with MetS and MetSO), but otherwise, there is no observed photochemical reactivity either in the presence or the absence of dioxygen.

Altogether, the photochemistry of MetS and its oxidized forms, as summarized in Scheme 5, provides some useful insights into the mechanisms of photoredox processes of potential relevance to cataract formation. As has been postulated, the presence of dioxygen is clearly established in the amino acid model; only under aerobic conditions is photoactivated MetS oxidation observed. In addition, we note that photoirradiation of MetSO can actually *repair oxidative damage* and reduce the sulfoxide back to MetS. However, this mechanism is only effective under anaerobic conditions; in the presence of O_2, reduction *and* oxidation both occur, although it appears that these do not result from bimolecular disproportionation. Oxidation results in the formation of $MetSO_2$, which is both photochemically and enigmatically inert toward reduction back to MetSO and/or MetS. The formation of the sulfone may ultimately be the most detrimental product of photooxidation, as its formation is both facile and irreversible. We are currently investigating the specifics of the photochemistry of α-crystallin to determine whether its behavior is similar and to provide additional insights into the photochemistry related to cataract formation.

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Supporting Information Available: S K-edge XAS spectrum of $NaCH_3SO_2$ (S1), background photoreduction of MetSO induced by the X-ray beam (S2), ^1H NMR spectra of MetS, MetSO, and $MetSO_2$ (S3), results of target transformation (S4), S K-edge XAS spectra of photolysis of $MetSO_2$ under aerobic conditions (S5), UV−vis spectra of MetS, MetSO, and $MetSO_2$ (S6), and S K-edge XAS spectra of photolysis of DMSO under anaerobic conditions (S7). This material is available free of charge via the Internet at http://pubs.acs.org.

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