Reaction of a Coordinated Cysteinato Ligand with Singlet Oxygen: Photooxidation of (Cysteinato-*N,S*)bis(ethylenediamine)cobalt(III)

Cesar Galvez, David G. Ho, Armin Azod, and Matthias Selke*

Department of Chemistry and Biochemistry
California State University, Los Angeles
Los Angeles, California 90032

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Singlet oxygen, the $^1\Delta_g$ state of the dioxygen molecule, is capable of damaging nucleic acids, proteins, and lipids in the cellular environment.[1] Thus, reactions of singlet oxygen with nucleotide bases and amino acids have been the subject of intensive research for several decades.[2] In many cases, highly unstable primary products have been directly observed or trapped.[3] Insight into the mechanism of the photooxidation of such molecules has also been obtained from a large amount of kinetic data collected for many different systems.[4] Yet amino acids also function as ligands at the active site of many enzymes. Almost nothing is known about the reactivity of singlet oxygen with such metal-coordinated amino acids. Free cysteine is known to react with singlet oxygen, leading to the formation of disulfide bonds.[5] There have been an increasing number of reports of oxidative damage to sulfur-rich metalloenzymes.[6] Oxidized cysteinato ligands have also been observed in a recent crystal structure of nitrile hydratase, a microbal enzyme that contains Fe(III) or Co-(III) at its active site.[7] However, despite the abundance of S-coordinated cysteinato ligands in enzymatic systems and the obvious biological importance of oxidative damage to such ligands, there have been, to the best of our knowledge, no prior reports of reactions of singlet oxygen with S-coordinated cysteinato ligands. We now report that singlet oxygen cleanly oxidizes the thiolato moiety of the complex $[Co(III)(en)_2(S\text{-}cys)]^+(BF_4)^-$ [8] (**1**) to the corresponding sulfenato complex. We also present kinetic and trapping data that provides strong evidence for a reaction mechanism analogous to the photooxidation of organic sulfides.

Photooxidation of $[Co(en)_2(S\text{-}cys)]^+(BF_4)^-$. The Co(III)−cysteinato complex $[Co(en)_2(S\text{-}cys)]^+(BF_4)^-$ (**1**) is unreactive with triplet oxygen in aqueous solution. However, reaction with singlet oxygen (sensitizer: methylene blue or rose bengal, solvent: water or 90% methanol/10% water mixture, Oriel tungsten−halogen 300 W lamp, cutoff filter at 492 nm) results in the formation of the corresponding sulfenato product $[Co(en)_2(SO\text{-}cys)]^+(BF_4)^-$ (**2**).[9]

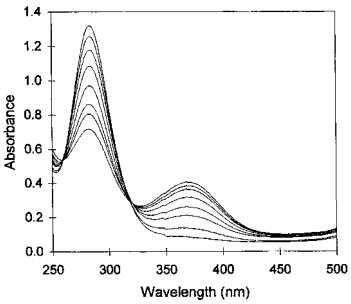
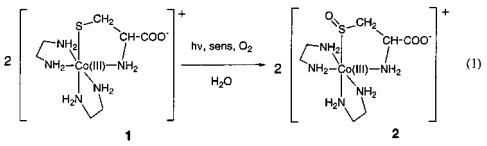


Figure 1. Change in the UV/vis spectrum during the photooxidation of $[Co(en)_2(S\text{-}cys)]^+(BF_4)^-$ (**1**). The peak at 287 nm is due to complex **1**, while that at 370 nm is due to the sulfenato complex **2**.



The reaction can be carried out at pH 6−10. Product formation is almost quantitative under these conditions; however, strongly acidic or basic conditions lead to extensive decomposition. The reaction has been followed by UV/vis spectroscopy (Figure 1), and by ^{1}H NMR spectroscopy. Both the UV/vis data and the NMR data are identical with the literature values of **2**.[8] Control experiments demonstrate that the reaction is not a self-sensitzed photooxygenation, as no reaction takes place upon irradition under an oxygen atmosphere without the presence of the sensitizer. The reaction is faster in D_2O than in H_2O, consistent with singlet oxygen being the oxidant. For example, a 0.1 mM sample of **1** reacts approximately 20 times faster in D_2O than H_2O under otherwise identical conditions.[11] No C−S bond cleavage products are observed. Prolonged reaction with singlet oxygen (irradiation for 1 h or more) leads to formation of small amounts of the corresponding sulfinato complex $[Co(en)_2(SO_2\text{-}cys)]^+(BF_4)^-$ (**3**) after all starting material has been converted to the sulfenato complex **2**. Small amounts of the sulfinato complex **3** are also observed within very short irradiation times at low substrate concentration (∼2 min irradiation at [**1**] < 0.1 mmol) However, under those conditions, addition of a large excess (∼100 fold) of DMSO completely suppresses the formation of **3**, and only the sulfenato product **2** is obtained.

Kinetics of Reaction of $[Co(en)_2(S\text{-}cys)]^+(BF_4)^-$ with Singlet Oxygen. Singlet oxygen can both be physically quenched by (k_q) or chemically react with (k_r) substrates. The total rate of removal of singlet oxygen by complex **1** (k_T) has been measured by a

(1) For recent examples, see: (a) Michaeli, A.; Feitelson, J. *Photochem. Photobiol.* **1997**, *65*, 309. (b) Cadet, J.; Berger, M.; Douki, T.; Morin, B.; Raoul, S.; Ravannat, J. L.; Spinelli, S. *Biol. Chem.* **1997**, *378*, 1275.

(2) (a) Cadet, J.; Vigny, P. In *The Photochemistry of Nucleic Acids*; Morrison, H., Ed.; John Wiley and Sons: New York, 1990; pp 1−272. (b) Foote, C. S.; Clennan, E. L. In *Active Oxygen in Chemistry*; Foote, C. S., Valentine, J. S., Greenberg, A., Liebman, J. F., Eds.; Blackie Academic and Professional (Chapman & Hall): New York, 1995; Chapter 4.

(3) For example, the highly unstable primary endoperoxide product in the photooxidation of a guansosine derivative has been directly observed: Sheu, C.; Foote, C. S. *J. Am. Chem. Soc.* **1993**, *115*, 10446.

(4) Wilkinson, F.; Helman, W. P.; Ross, A. B. *J. Phys. Chem. Ref. Data* **1995**, *24*, 663.

(5) Rougee, M.; Benasson, R. V.; Land, E. J.; Pariente, R. *Photochem. Photobiol*. **1988**, 485.

(6) See, for example: (a) Wilcox et al. have recently reported that exposure of zinc fingers to oxygen leads to an increase in the mass by 32 mass units. Xu, Y.; Wilcox, D. E. *J. Am. Chem. Soc.* **1998**, *120*, 7375. (b) The activity of CO-dehydrogenase and [NiFe]-hydrogenase is inhibited by exposure to oxygen, apparently via oxidation of the thiolate ligand. Henderson, R. K.; Bouwman, E.; Spek, A. L.; Reedijk, J. *Inorg. Chem.* **1997**, *36*, 4616. (c) It has been suggested that vanadium(V) may inhibit protein tyrosine phosphatase by coordinating to the cysteine of the PTP active site, followed by oxidation of the thiolate moiety, possibly via a peroxovanadium complex. Huyer, G.; Liu, S.; Kelly, J.; Moffat, J.; Payette, P.; Kennedy, B.; Tsaprailis, G.; Gresser, M. J.; Ramachandran, C. *J. Biol. Chem.* **1997**, *272*, 843.

(7) Nagashima, S.; Nakasako, M.; Naoshi, D.; Tsujimura, M.; Takio, K.; Odaka, M.; Yohda, M.; Kamiya, N.; Endo, I. *Nat. Struct. Biol.* **1998**, *5*, 347.

(8) Sloan, C. P.; Krueger, J. H. *Inorg. Chem.* **1975**, *14*, 1481. Complex **1** was prepared as described in this paper, except that $NaBF_4$ was used instead of $NaClO_4$.

(9) The absolute configuration of the new chiral center formed at the sulfur could unfortunately not be determined, as there is a very rapid photocatalyzed interconversion of the *R*- and *S*-isomers, and complex **2** absorbs across almost the entire range of the visible spectrum. Even room light has been reported to be sufficient for this conversion.[10]

(10) Herting, D. L.; Sloan, C. P.; Cabral, A. W.; Krueger, J. H. *Inorg. Chem.* **1978**, *17*, 1649.

(11) The samples of Complex **1** used in our studies actually consist of 2:1 mixtures of the Λ and Δ isomers of **1**, as determined by ^{13}C NMR.[12] Following the reaction by ^{13}C NMR showed that both isomers react with singlet oxygen at the same rate. Also, the UV/vis experiments showed that the reaction rate does not change over the course of the reaction, again indicating that both isomers react at the same rate. Thus, no attempt to separate these isomers was undertaken during the kinetic studies described further below.

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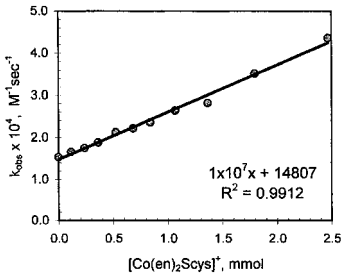


Figure 2. Singlet oxygen luminescence quenching by complex **1**.

singlet oxygen luminescence quenching experiment.[13] The combined rate of removal of singlet oxygen by physical quenching and chemical reaction thus obtained is $1.0 \pm 0.2 \times 10^7$ M^{-1} sec^{-1} (Figure 2) in D_2O at neutral pH. To measure the chemical reaction rate of **1** with singlet oxygen, competition experiments between **1** and the singlet oxygen acceptor 9,10-dimethylanthracene (DMA) were conducted in a 90% methanol/10% water mixture, at neutral pH. DMA is known to interact with singlet oxygen by chemical reaction only, with a rate constant of 2.4×10^7 M^{-1} sec^{-1} in methanol.[14] Loss of DMA and complex **1** was monitored spectrophotometrically,[15] and the results were fitted into the equation by Higgins et al.[16]

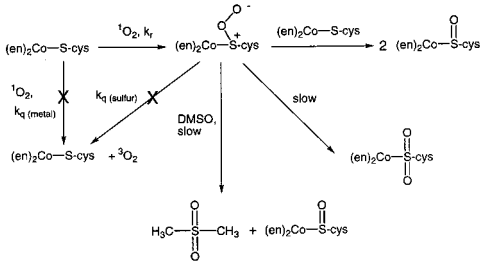
$$\frac{\log\{[\mathbf{1}]^f/[\mathbf{1}]^0\}}{\log\{[\mathrm{DMA}]^f/[\mathrm{DMA}]^0\}} = \frac{k_r(\mathbf{1})}{k_r(\mathrm{DMA})} \qquad (2)$$

The chemical reaction rate k_r of **1** thus obtained is $2.2 \pm 0.4 \times 10^7$ M^{-1} sec^{-1}, or, within limits of error, twice the rate of singlet oxygen removal by complex **1**, that is, $k_r = 2k_T$. This implies that two molecules of product are formed for every singlet oxygen molecule that is consumed and that there is no physical deactivation of singlet oxygen by complex **1**.

The kinetic data is consistent with the mechanism outlined in Scheme 1. This mechanism is very similar to that for the photooxidation of organic sulfides. Many transition metal complexes *physically* quench singlet oxygen,[4] including several Co−thiolato complexes.[17] However, the fact that the rate of oxidation of **1** is (within limits of error) twice the rate of singlet oxygen removal demonstrates that no physical quenching of $^1O_2$ by complex **1** occurs. The first putative intermediate in the photooxidation of organic sulfides is a persulfoxide.[18] Such intermediates have been trapped with DMSO.[19] In the photooxidation of organic sulfides, the intermolecular trapping of the persulfoxide prevents intramolecular rearrangement (probably via a *S*-hydroperoxysulfonium ylide[18]) to the sulfone. Our observation that formation of the sulfinate **3** is inhibited by addition of excess DMSO is thus additional evidence for the mechanism depicted in Scheme 1.

While there have been extensive studies of the reaction of organic sulfides with singlet oxygen, there have been very few reports of the photooxidation of metal thiolates. Darensbourg and co-workers have elucidated the mechanism of the oxidation of bisthiolato ligands coordinated to nickel complexes by triplet oxygen.[20] In addition, it has been suggested that the reaction of such nickel thiolates with singlet oxygen[20e−f] (as well as related

Scheme 1. Reaction Pathways of $[Co(en)_2(S\text{-}cys)]^+(BF_4)^-$ (**1**) with Singlet Oxygen



Pt[21] and Pd[20f,22] complexes) follows the same mechanistic pathway as the reaction of organic sulfides with $^1O_2$. However, except for the case of an apparently self-sensitized Pt system,[23] no kinetic data in support of this hypothesis has thus far been presented, and attempts to trap intermediates with diphenyl sulfoxide have not been successful.[20f] Our data provide strong evidence that metal thiolates, including S-bound cysteinato ligands, can react with singlet oxygen via a mechanism that is analogous to the photooxidation of organic sulfides.

Reaction rates of organic sulfides with singlet oxygen are influenced by the steric bulk of the alkyl groups. However, despite the bulky octahedral complex to which the reactive sulfur of complex **1** is coordinated, the rate of removal of singlet oxygen by the cysteinato ligand is approximately 5 times the rate of photooxidation of methylphenyl sulfide in methanol,[24] and about as large as that of ethyl sulfide in methanol.[25] Furthermore, formation of the sulfinate is observed only at low substrate concentration. Thus, at relatively high concentrations of **1**, intermolecular reaction of the persulfoxide intermediate with unreacted starting material is still faster than collapse of this intermediate to the sulfinate, despite the large steric bulk of the octahedral Co complex. On the other hand, in a biological environment, the concentration of cysteinato ligands will usually be low enough that sulfinate formation will be the major pathway, except at sites where several cysteinato ligands are in close proximity and attack of the persulfoxide on a nearby cysteinato ligand is possible.

The large chemical reaction rate constant and the absence of physical quenching of singlet oxygen by the metal center indicate S-coordinated cysteinato ligands may generally be susceptible to oxidation by singlet oxygen. Further experiments to establish the effects of the particular metal and its oxidation state on the photooxidation of thiolato ligands are in progress.

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