Interaction of Arsenate with the Molybdenum Site of Sulfite Oxidase

Graham N. George,*,† Robert M. Garrett,‡ Tyler Graf,‡
Roger C. Prince,§ and K. V. Rajagopalan‡

Stanford Synchrotron Radiation Laboratory, SLAC
Stanford University, P.O. Box 4349, MS 69
Stanford, California 94309
Department of Biochemistry, Duke University
Durham, North Carolina 27710
Exxon Research and Engineering Company
Annandale, New Jersey 08801

Received October 6, 1997

Sulfite oxidase catalyzes the physiologically vital oxidation of sulfite to sulfate.[1] It contains molybdenum associated with a single molybdopterin dithiolene cofactor and a cytochrome *b*-type heme.[1,2] The two-electron oxidation of sulfite to sulfate occurs at the molybdenum site, which is reduced from Mo(VI) to Mo(IV), and the catalytic cycle is completed by two one-electron oxidations to the cytochrome *b* site.[3] A variety of anions are competitive inhibitors of sulfite oxidase.[4] The best studied is phosphate, which forms a characteristic Mo(V) electron paramagnetic resonance (EPR) signal called the phosphate signal. This signal shows hyperfine coupling to the $I = {}^1/_2$ ^{31}P nucleus,[5,6] and to ^{17}O of enriched phosphate,[7] indicating molybdenum−phosphate coordination. It also lacks the strongly coupled exchangeable protons[5] of the other Mo(V) EPR signals.[8] Gutteridge et al.[7] showed that the strongly coupled ^{17}O observed in the Mo(V) EPR of ^{17}O-enriched enzyme is lost upon formation of the phosphate complex, suggesting that an −OH ligand of molybdenum is displaced by binding of the anion.[7] The structure of the complex is of significant interest because it is likely to be structurally analogous to the sulfate complex with reduced enzyme formed after catalytic sulfite oxidation. We report herein a study of reduced sulfite oxidase complexed with the phosphate analogue arsenate, using both EPR and extended X-ray absorption fine structure (EXAFS) spectroscopies.

Figure 1 shows the effects of phosphate and arsenate upon the X-band Mo(V) EPR spectrum[9] of human sulfite oxidase. The arsenate signal (Figure 1c) has *g* values to the phosphate signal (Figure 1b), but with additional resolved structure due to ligand hyperfine coupling. Only very subtle sharpening of the arsenate EPR signal was observed in ^2H_2O buffer, as was previously observed for the phosphate signal,[5] indicating the absence of strongly coupled exchangeable protons in the signal-giving species. For the phosphate signal, ^{31}P hyperfine is weak[5,6] and has only been observed in higher derivative X-band EPR[5] or by sophisticated pulsed techniques.[6] Arsenic (100% ^{75}As $I = {}^3/_2$) is expected from theory to have hyperfine couplings similar in size to those of ^{31}P.[10] However ^{75}As also has a significant nuclear electric quadrupole moment (0.290 barns[11]), and complication of the EPR spectrum due to nuclear electric quadrupole coupling is anticipated.[12] The quadrupole coupling is directly proportional to the electric field gradient at the quadrupolar nucleus, arising from the valence electronic structure and from the influence of nearby atoms. Complexed arsenate is expected to be somewhat distorted from ideal tetrahedral geometry, with bond lengths varying from approximately 1.67 Å for As═O to 1.73 Å for As−OH[13] or Mo−O−As ligation.[14] In the EPR signal, we can therefore expect a significantly asymmetric nuclear electric quadrupole coupling from the distorted arsenic ligand field. Figure 1d shows a simulation,[16] including quadrupole effects, of the Mo(V) arsenate EPR spectrum.[17]

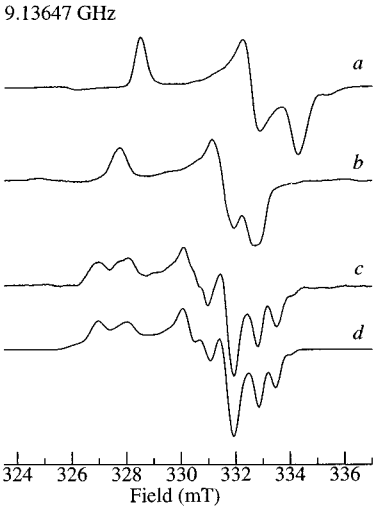

Figure 1. Mo(V) EPR spectra of sulfite-reduced human sulfite oxidase in ^2H_2O (a) in the absence of added anions at pH 9.0, (b) in the presence of 0.1 M phosphate at pH 6.5, and (c) in the presence of 0.1 M arsenate at pH 6.5. Trace d shows a simulation of c.[17]

The Mo(V) EPR signal of Figure 1c integrates[18] to approximately 58% Mo(V), and the sample is likely to be a mixture of Mo(V) and Mo(IV). While interpretation of EXAFS spectra

† Stanford University.
‡ Duke University.
§ Exxon Research and Engineering Co.

(1) (a) McLeod, R. M.; Farkas, W.; Fridovitch, I.; Handler, P. *J. Biol. Chem.* **1961**, *236*, 1841−1852. (b) Cohen, H. L.; Betcher-Lange, S.; Kessler, D. L.; Rajagopalan, K. V. *J. Biol. Chem.* **1972**, *247*, 7759−7766.

(2) Kisker, C.; Schindelin, H.; Pacheco, A.; Wehbi, W. A.; Garrett, R. M.; Rajagopalan, K. V.; Enemark, J. E.; Rees, D. C. *Cell* **1997**, *91*, 1−20.

(3) (a) Johnson, J. L.; Rajagopalan, K. V. *J. Biol. Chem.* **1977**, *252*, 2017−2025. (b) Kipke, C. A.; Cusanovitch, M. A.; Tollin, G.; Sunde, R. A.; Enemark, J. H. *Biochemistry* **1988**, *27*, 2918−2926. (c) Sullivan, E. P.; Hazzard, J. T.; Tollin, G.; Enemark, J. H. *Biochemistry* **1993** *32*, 12465−12470.

(4) Kessler, D. L.; Rajagopalan, K. V. *Biochim. Biophys. Acta* **1974**, *370*, 389−398.

(5) George, G. N.; Prince, R. C.; Kipke, C. A.; Sunde, R. A.; Enemark, J. E. *Biochem. J.* **1988**, *256*, 307−309.

(6) Pacheco, A.; Basu, P.; Borbat, P.; Raitsimring, A. M.; Enemark, J. E. *Inorg. Chem.* **1996**, *35*, 7001−7008.

(7) Gutteridge, S.; Lamy, T. M.; Bray, R. C. *Biochem. J.* **1980**, *191*, 285−288.

(8) (a) Lamy, M. T.; Gutteridge, S.; Bray, R. C. *Biochem. J.* **1980**, *185*, 397−403. (b) George, G. N. *J. Magn. Reson.* **1985**, *64*, 384−394.

(9) EPR spectroscopy used a Varian E109 instrument, with 0.1-mT modulation amplitude, 10-mW applied power, and the sample at 150 K. Recombinant human sulfite oxidase was purified as previously described (Garrett, R. M.; Rajagopalan, K. V. *J. Biol. Chem.* **1996**, *271*, 7387−7391). Samples for EPR spectroscopy and for XAS (0.1 and 0.5 mM Mo, respectively) in 50 mM bis-tris−propane buffer at pH 6.5 were reduced with 1 mM sulfite for 30 s.

(10) Goodman, B. A.; Raynor, J. B. *Adv. Inorg. Chem. Radiochem.* **1970**, *13*, 135−362.

(11) Enge, H. *Introduction to Nuclear Physics;* Addison-Wesley: Reading MA, 1974; Appendix 6.

(12) Hyperfine and nuclear electric quadrupole couplings to ^{75}As have been reported for arsenite-inhibited xanthine and aldehyde oxidases [George, G. N.; Bray, R. C. *Biochemistry* **1983**, *22*, 1013−1021. Turner, N. A.; Doyle, W. A.; Bray, R. C. *Eur. J. Biochem.* **1995**, *232*, 646−657].

(13) Wells, A. F. *Structural Inorganic Chemistry*, 5th ed.; Clarendon Press: Oxford, U.K., 1982.

(14) For example: (a) Hsu, K.-F.; Wang, S.-L. *Inorg. Chem.* **1997**, *36*, 3049−3054. (b) Ribas, J.; Poilblanc, R.; Sourisseau, C.; Solans, X.; Luis Brisano, J.; Miravitlles, C. *Trans. Met. Chem.* **1983**, *8*, 244−250.

(15) Computer simulation of EPR spectra was performed by fitting spectra to the $S = {}^1/_2$ spin Hamiltonian: $H = \beta_e\mathbf{B}\cdot\mathbf{g}\cdot\mathbf{S} + h\mathbf{S}\cdot\mathbf{A}\cdot\mathbf{I} + h\mathbf{I}\cdot\mathbf{P}\cdot\mathbf{I} - \beta_n g_n \mathbf{B}\cdot\mathbf{I}$ All symbols have their usual meanings[10] and a value of 0.959 was used for g_n.[11] Simulations were computed using the program QPOW of Belford and co-workers[16] modified to allow fitting to experimental data.

(16) (a) Nilges, M. J. Ph.D. Thesis, University of Illinois, 1979. (b) Maurice, A. M. Ph.D. Thesis, University of Illinois, 1980. (c) Belford R. L.; Nilges, M. J. Proceedings of the Symposium on EPR Spectroscopy, Rocky Mountain Conference on Analytical Chemistry, Denver, CO, 1979.

S0002-7863(97)03473-2 CCC: $15.00
Published on Web 04/28/1998

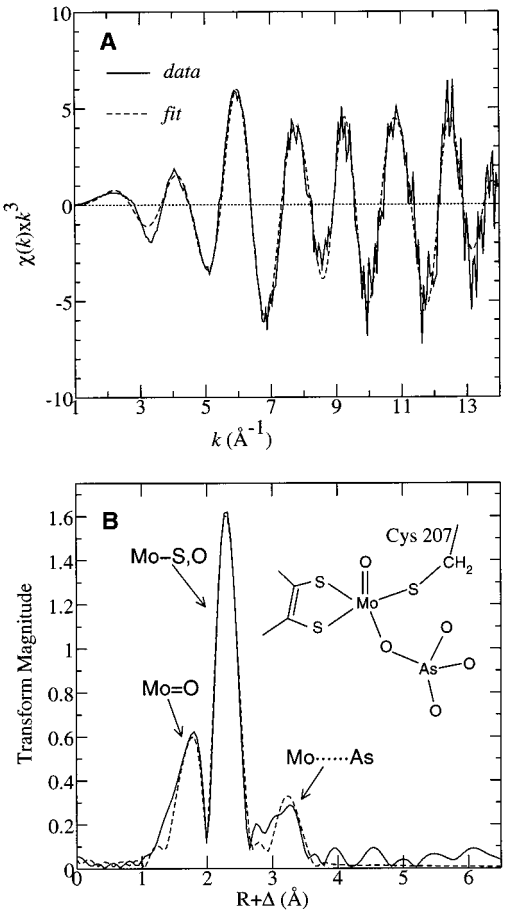

Figure 2. (A) Mo K-edge EXAFS and (B) EXAFS Fourier transform (Mo−S phase-corrected) of sulfite-reduced sulfite oxidase in the presence of 0.1 M arsenate. The solid lines show the experimental data and the broken lines the best fit: three Mo−S at 2.363(6) Å, $\sigma^2 = 0.0038(5)$ Å^2, one Mo═O at 1.695(4) Å, $\sigma^2 = 0.0016(3)$ Å^2, one Mo−O at 2.26(3) Å, $\sigma^2 = 0.0021(5)$ Å^2, and 0.6 Mo···As at 3.203(6) Å, $\sigma^2 = 0.0025(5)$ Å^2, where σ^2 are Debye−Waller factors and the parenthetical values are estimated standard deviations (precisions) from the diagonal elements of the covariance matrix. Accuracies are expected to be poorer than precisions (for bond lengths < ±0.02 Å). The inset in B is a postulated structure for the complex; we note that no geometrical information is available from our EXAFS analysis.

of mixtures can be problematic, the primary goal of this work was to detect Mo···As interactions and we have therefore examined the Mo K-edge EXAFS of arsenate-complexed reduced sulfite oxidase.[19] Figure 2 shows the Mo K-edge EXAFS and Fourier transform together with the best fit, which is given by three Mo−S, one Mo═O, one Mo−O, and one Mo···As interaction. The Mo−S coordination number of three is as expected from previous work[2,21,22] with one sulfur ligand originating from Cys 207[2,22,23] and two more from the cofactor dithiolene.[2,22] Similarly, a single Mo═O and at least one Mo−O are expected for reduced enzyme[21] and the Mo═O coordination number of one argues for minimal presence of the dioxo Mo(VI) enzyme. The data did not allow inclusion of yet another Mo−O interaction,[24] and we conclude that the site is five-coordinate, with the structure of the type shown in Figure 2B. The best fit coordination number for the Mo···As interaction was 0.5−0.6. While this is consistent with only the Mo(V) form being complexed, we note that EXAFS-derived coordination numbers for distant ligands such as the Mo···As lack sufficient accuracy to be certain of this. In any case, first-shell structural differences between the Mo(V) and Mo(IV) sites must be small, as the low values of the Debye−Waller factors do not indicate significant heterogeneity in metal−ligand bond lengths. Assuming that the 2.26 Å Mo−O is that ligated to arsenic (see Figure 3) and assuming an O−As distance of 1.72 Å,[14] then we can calculate an approximate Mo−O−As bond angle of 130°, which is a chemically reasonable value.[14] It has been suggested that phosphate binds as a bidentate ligand.[7] Such ligation now seems unlikely; assuming an O−As−O bond angle of 109°,[13] we calculate a closest approach to molybdenum of a second arsenic-bound oxygen of 3 Å for the arsenate complex.

By comparing the hyperfine couplings of ^{31}P and ^{75}As of the phosphate and arsenate Mo(V) EPR signals, we find that the coupling to arsenic is approximately twice as large. This might, at first sight, argue for different structures for the arsenate and phosphate complexes; however, the isotropic component of the ^{31}P hyperfine coupling in the phosphate signal of site-directed mutant sulfite oxidases can be much larger,[25] indicating that it can be significantly affected by subtle geometric changes. From this consideration and the similarity in the g values (Figure 1), we believe that the phosphate and arsenate complexes are very similar. From analysis of the anisotropic component of the ^{31}P hyperfine coupling, Pacheo et al.[6] estimated a Mo···P distance of 3.2−3.3 Å for the sulfite oxidase phosphate signal-giving species. This is in excellent accord with our Mo···As distance of 3.20 Å. Pacheo et al. additionally estimate a Mo−O bond length (assuming chemically reasonable phosphate bond lengths and angles) of a monodentate phosphate ligand to be approximately 2.25 Å, also in good agreement with our measurement of 2.26 Å for what is likely the analogous bond in the arsenate complex. As indicated above, the arsenate and phosphate complexes are of particular interest in that they are likely to be structurally analogous to the sulfate complex with reduced enzyme which will be formed after catalytic oxidation of sulfite. By analogy, we therefore expect sulfate in such a complex to be coordinated to molybdenum as a monodentate ligand.[26]

Acknowledgment. SSRL is funded by DOE BES, with further support by DOE OBER and NIH. Research at Duke University was supported by NIH GM00091 (K.V.R.). We thank Dr. David Lowe for recording 35-GHz EPR spectra and Dr. Ingrid Pickering for assistance with EXAFS data collection.

JA973473N

(17) EPR parameters were $g_{x,y,z} = 1.9636, 1.9701, 1.9933$, $[^{75}\text{As}]A_{x,y,z} = 12.4, 24.9, 15.9$ MHz, and angles of noncollinearity[12] (constrained to be identical for **A** and **P**) α, β, γ = 49.4, 0.0, 14.2°, with $[^{75}\text{As}]P_{x,y,z} = -7.8, 3.3, +4.5$ MHz. The quadrupole coupling parameters are within the expected range for arsenate (Katowski, P.; Mackowiak, J. *Magn. Reson. Chem.* **1995**, *33*, 848−851). Satellite features due to hyperfine splittings of the 15% ^{95}Mo and 10% ^{97}Mo (both $I = 5/2$; e.g. George, G. N. and Bray, R. C. *Biochemistry* **1988**, *27*, 3603−3609) were not included. The validity of the simulation was checked with 35-GHz EPR (not illustrated).

(18) Integrations were performed using a 1 mM CuEDTA standard (Aasa, R.; Vänngård, T. *J. Chem. Phys.* **1970**, *52*, 1612−1613).

(19) XAS was carried out on SSRL beamline 7−3.[22] EXAFS oscillations $\chi(k)$ were quantitatively analyzed with EXAFSPAK (obtained by contacting the authors) using ab initio phase and amplitude functions generated with *Feff* V7.02.[20] No smoothing, filtering, or related manipulation was performed upon the data.

(20) (a) Rehr, J. J.; Mustre de Leon, J.; Zabinsky, S. I.; Albers, R. C. *J. Am. Chem. Soc.* **1991**, *113*, 5135−5140. (b) Mustre de Leon, J.; Rehr, J. J.; Zabinsky, S. I.; Albers, R. C. *Phys. Rev.* **1991**, *B44*, 4146−4156.

(21) George, G. N.; Kipke, C. A.; Prince, R. C.; Sunde, R. A.; Enemark, J. H.; Cramer, S. P. *Biochemistry* **1989**, *28*, 5075−5080.

(22) George, G. N.; Garrett, R. M.; Prince, R. C.; Rajagopalan, K. V. *J. Am. Chem. Soc.* **1996**, *118*, 8588−8592.

(23) Garrett, R. M.; Rajagopalan, K. V. *J. Biol. Chem.* **1996**, *271*, 7387−7391.

(24) Refinements including an additional Mo−O yielded very large Debye−Waller factors that effectively removed this Mo−O EXAFS.

(25) George, G. N., Garrett, R. M.; Graf, T.; Prince, R. C.; Rajagopalan K. V. Unpublished work.

(26) Formation of a monodentate sulfate complex might proceed via a bidentate intermediate if sulfite is directly coordinated to molybdenum in the Michaelis complex.