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X-ray Absorption Spectroscopic Characterization of the Molybdenum Site of *Escherichia coli* Dimethyl Sulfoxide Reductase

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Structural studies of dimethyl sulfoxide (DMSO) reductases were hampered by modification of the active site during purification. We report an X-ray absorption spectroscopic analysis of the molybdenum active site of *Escherichia coli* DMSO reductase contained within its native membranes. The enzyme in these preparations is expected to be very close to the form found in vivo. The oxidized active site was found to have four Mo–S ligands at 2.43 Å, one Mo═O at 1.71 Å, and a longer Mo–O at 1.90 Å. We conclude that the oxidized enzyme is a monooxomolybdenum(VI) species coordinated by two molybdopterin dithiolenes and a serine. The bond lengths determined for *E. coli* DMSO reductase are very similar to those determined for the well-characterized *Rhodobacter sphaeroides* DMSO reductase, suggesting similar active site structures for the two enzymes. Furthermore, our results suggest that the form found in vivo is the monooxobis(molybdopterin) species.

The dimethyl sulfoxide (DMSO) reductase of *Escherichia coli* is a membrane-bound multiprotein complex that provides the terminal step in the electron-transfer chain when the organism is growing on DMSO.[1] It is a complex molybdenum and Fe−S cluster-containing enzyme that is bound to the plasma membrane. The enzyme consists of three subunits. The largest, DmsA, contains the molybdenum active site of DMSO reduction, DmsB contains four [4Fe−4S] clusters and functions in electron transfer, while DmsC anchors the DmsAB subunits to the membrane.[1] The reduction of DMSO is catalyzed at the molybdenum site, which is associated with two molybdopterin guanine dinucleotide cofactors.[1]

$$\text{Enz-Mo}^{IV} + (CH_3)_2SO + 2H^+ \rightarrow \text{Enz-Mo}^{VI} + (CH_3)_2S + H_2O$$

DmsA is related to the structurally well-characterized monomeric soluble periplasmic DMSO reductases from *Rhodobacter sphaeroides*[2−4] and *Rhodobacter capsulatus*,[5] but to date, there is no direct structural information on the active site of *E. coli* DMSO reductase. Early structural studies of the *Rhodobacter* DMSO reductases[6−8] suffered from problems due to modifications of the active site by different sample treatments. In particular, three early crystal structures from three different groups[6−8] gave conclusions that were in conflict both with results from spectroscopy[2,9] and with each other. It is now generally agreed that the root of this confusion was cocrystallization of multiple forms of the active site, consisting of active and inactive species, arising from different sample treatments.[3−5] Two structures of the oxidized Mo^{VI} active site are found, one a six-coordinate monooxo species with serine oxygen and four sulfurs from two coordinated molybdopterin dithiolene cofactors[3,4] and the other a five-coordinate dioxo species with a serine ligand and only one of the two active site dithiolene cofactors bound.[4,7] Because the former structure is that observed both in solution and following catalytic turnover,[3] it is generally supposed that this corresponds to a physiologically relevant active site.

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One advantage of X-ray absorption spectroscopy (XAS) is that it has no restrictions on the sample state. We present herein an XAS study[10] of the molybdenum site of *E. coli* DMSO reductase contained within its native membrane,[16] which has had only minimal preparation and should therefore be close to the form found in vivo. The concentration of molybdenum in these samples is approximately 40 μM, which is challenging for XAS. However, recent advances in sensitivity[18] allow us to collect adequate data even at such low concentrations.

Figure 1 shows the Mo K X-ray absorption near-edge spectrum of membranous DmsABC compared with the spectra of prototypical members of the three families of molybdenum enzymes. As expected, the near-edge spectrum is distinct from sulfite oxidase and xanthine oxidase but closely resembles that of *R. sphaeroides* DMSO reductase (Figure 1). The extended X-ray absorption fine structure (EXAFS) spectrum of DmsABC is shown in Figure 2, together with best fits and the corresponding Fourier transforms, and the parameters obtained from curve fitting are summarized in Table 1.

The curve-fitting analysis clearly indicates the presence of a single Mo═O group at 1.71 Å, four sulfurs at 2.43 Å, and a longer oxygen at 1.90 Å. The four sulfur atoms can be attributed to chelation by the dithiolene moieties of the two molybdenum cofactors and the longer oxygen to a conserved serine previously suggested to be a ligand of molybdenum (Ser[176]).[17] Of the three interactions included in our analysis, Mo−O is the least definitively determined because it does not give rise to a clearly resolved peak in the Fourier transform. Omitting the Mo−O interaction in the

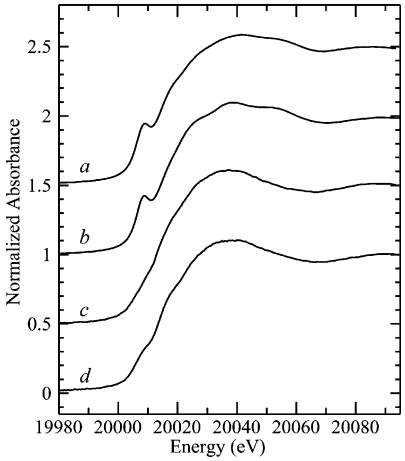

Figure 1. X-ray absorption near-edge spectra of DmsABC compared with prototypical members of the three families of molybdenum enzymes: (a) sulfite oxidase; (b) xanthine oxidase; (c) *R. sphaeroides* DMSO reductase; (d) DmsABC.

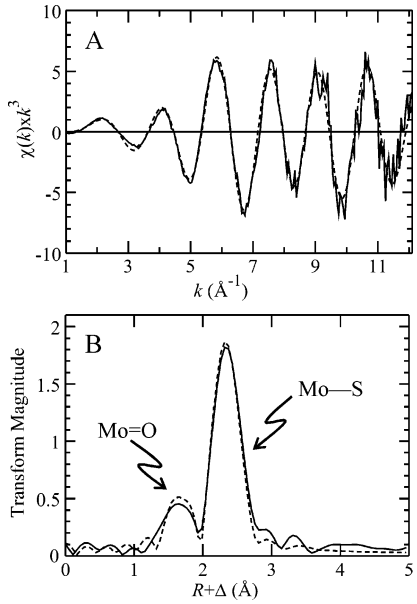

Figure 2. (A) Mo K-edge EXAFS spectrum of membranous DmsABC (solid line) plus best fit (broken line). (B) Corresponding Fourier transforms phase-corrected for Mo−S backscattering.

Table 1. EXAFS Curve-Fitting Parameters for *E. coli* DmsABC[a]

interaction	*N*	*R*	σ^2	ΔE_0	*F*
Mo−O	1	1.711(3)	0.0017(1)	−14.3(5)	0.281
Mo−S	4	2.429(1)	0.0043(1)		
Mo−O	1	1.902(9)	0.0042(4)		

[a] Coordination numbers *N*, interatomic distances *R* (Å), Debye−Waller factors σ^2 (Å^2), and threshold energy shifts ΔE_0 (eV). Values in parentheses are the estimated standard deviations (precisions) obtained from the diagonal elements of the covariance matrix. Accuracies are greater than these values and are generally accepted to be ±0.02 Å for bond lengths and ±20% for coordination numbers and Debye−Waller factors. The fit-error function *F* is as previously defined.[3]

(10) X-ray absorption spectroscopic (XAS) measurements were conducted at the Stanford Synchrotron Radiation Laboratory with the SPEAR 3 storage ring containing between 85 and 100 mA at 3.0 GeV. Data were collected on the structural molecular biology XAS beamline 9-3 with a wiggler field of 2 T and employing a Si(220) double-crystal monochromator. Beamline 9-3 is equipped with a rhodium-coated vertical collimating mirror upstream of the monochromator and a downstream bent-cylindrical focusing mirror (also rhodium-coated). Harmonic rejection was accomplished by setting the cutoff angle of the mirrors to 23 keV. Incident and transmitted X-ray intensities were monitored using argon-filled ionization chambers, and X-ray absorption was measured as the Mo Kα fluorescence excitation spectrum using an array of 30 germanium detectors.[11] During data collection, samples were maintained at a temperature of approximately 10 K using an Oxford Instruments liquid-helium-flow cryostat. Ten 40-min scans were averaged so as to obtain acceptable signal-to-noise ratios, and the energy was calibrated by reference to the absorption of a molybdenum metal foil measured simultaneously with each scan, assuming a lowest energy inflection point of 20 003.9 eV. The energy threshold of the EXAFS oscillations was assumed to be 20 025.0 eV. Other experimental details were as previously described.[12] EXAFS oscillations $\chi(k)$ were quantitatively analyzed using the EXAFSPAK program suite [http://ssrl.slac.stanford.edu/exafspak.html] employing ab inito phase and amplitude functions computed using the program *FEFF*, version 8.25.[13,14] No smoothing, filtering, or related operations were performed on the data. Background subtraction was accomplished using a specially written program *BACKSUB* similar to that described by Weng and co-workers.[15]

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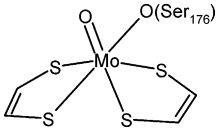

Figure 3. Postulated structure for the molybdenum active site of DmsABC. Only the dithiolene carbons of the two molybdopterin cofactors are shown.

EXAFS curve-fitting analysis results in an increase in the fit-error function from 0.218 to 0.322, which corresponds to a significantly worse fit because we estimate that close to 0.2 of the error is contributed by high-frequency noise.[19] Thus, our analysis indicates a structure similar to that determined for active *R. sphaeroides* DMSO reductase, which gave almost identical Mo−S and Mo−O bond lengths of 2.44 and 1.92 Å, respectively, and a marginally shorter Mo═O bond length of 1.68 Å.[2,3] All three interactions are close to or within the generally accepted EXAFS accuracy of ± 0.02 Å. Background subtraction of EXAFS for low-concentration samples is significantly more challenging than samples at higher concentrations,[15] and although considerable care was taken, some slight distortion (lengthening) of the Mo═O bond might arise from artifacts of background subtraction. Thus, we conclude that, within the uncertainties of our technique, the observed bond lengths for the DMSO reductases from *R. sphaeroides* and *E. coli* are identical.

Figure 3 shows a postulated local structure for the molybdenum active site of oxidized DmsABC with a geometry similar to that observed for *R. sphaeroides* DMSO reductase.[4] While geometrical information is not directly available from our EXAFS analysis, the absence of any trans-effect elongation of the Mo−S ligands argues for nonoctahedral geometry, and the similarity of the near-edge spectra (Figure 1) strongly argues for geometrical similarity between the two enzyme active sites.

The catalytic activities of *Rhodobacter* and *E. coli* DMSO reductases differ in having opposite enantioselectivity; *R. capsulatus* DMSO reductase selectively reduces the *S* enantiomer of methyl-*p*-tolyl sulfoxide, while *E. coli* DMSO reductase preferentially reduces the *R* enantiomer.[20] Our results indicate that this difference in enantioselectivity is not reflected by any EXAFS-detectable changes in the bond lengths of ligands that are coordinated directly to molybdenum. However, geometrical differences between the two enzyme active sites are certainly possible, for example, opposite geometrical arrangements of Mo═O and Mo−O(Ser) ligands for the *E. coli* and *Rhodobacter* enzymes. Such differences in coordination would not be reflected by any changes in the near-edge spectra (i.e., spectra of enantiomers would appear identical).

In summary, we have used XAS to investigate the active site structure of *E. coli* DMSO reductase contained within its native membranes. We find that the oxidized active site is a monooxo species with four sulfurs coordinated, plus a longer oxygen, and that it is strikingly similar to that postulated for *R. sphaeroides* DMSO reductase. Furthermore, our results also suggest that the same form is likely to exist in vivo.

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