A Model for Ferricyanide-Inhibited Sulfite Oxidase

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Sulfite oxidase is a pyranopterin molybdenum enzyme which catalyzes the physiologically important oxidation of sulfite to sulfate, the terminal step in the oxidative degradation of cysteine and methionine.[1−4] The crystal structure of chicken liver sulfite oxidase has been solved to 1.9 Å resolution[5] and, when interpreted in the context of a recent XAS study,[6] allows a detailed description of the active site stereochemistry. The geometry of the catalytically competent Mo(VI) active site is square pyramidal, with the molybdenum coordinated by two terminal oxo ligands, two sulfur donors from the ene-1,2-dithiolate side chain of the pyranopterin, and an additional sulfur donor from a cysteine residue. The two-electron oxidation of sulfite occurs via formal oxygen atom transfer,[7] resulting in the production of two reducing equivalents which are transferred sequentially from the mono-oxo Mo(IV) site to an exogenous cytochrome *c* acceptor via an endogenous *b*-type heme. Interestingly, incubation of active enzyme with ferricyanide results in the two-electron oxidation of the pyranopterin cofactor (Figure 1).[8] Although the enzyme is still competent to oxidize sulfite, egress of electrons from the molybdenum site is severely attenuated. This has led to two main hypotheses with regard to the role of the pyranopterin in sulfite: cytochrome *c* activity, namely, acting as an electron transfer conduit and modulating the molybdenum reduction potential.[3,8] The work described in this paper is directed toward discriminating between these two hypotheses and developing additional insight into the role of the pyranopterin under turnover conditions.

The compounds LMoO(qdt) (**1**)[9,10] and LMoO(bdt) (**2**)[11] were prepared according to literature precedent. We designed **1**, which possesses a pyrazine ring fused to a dithiolate chelate, as a first-generation model for ferricyanide-inhibited sulfite oxidase in order to understand the electronic origin of the inhibited enzyme's inability to reduce cytochrome *c*. Analogous to the (qdt) ligand in **1**, the pyrazine ring of the fully oxidized pyranopterin in inhibited enzyme is conjugated to an ene-1,2-dithiolate side chain (Figure 1c).[12] The pyranopterin in fully functional enzyme is present in the ring-closed tetrahydropterin form,[5,13] which contains a saturated pyrazine ring, instead of the ring-opened form (Figure 1a,b). Therefore, any differences in spectroscopic and electrochemical properties between **1** and **2** must reflect the effect of an oxidized pyrazine ring on the electronic structure and provide insight into differences between inhibited and functional enzyme. The 5 K, 7 T mull MCD spectra of **1** and **2** clearly indicate that the observed spectral features are essentially superimposable,

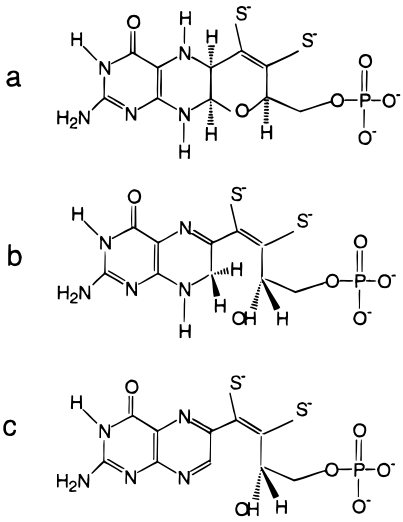
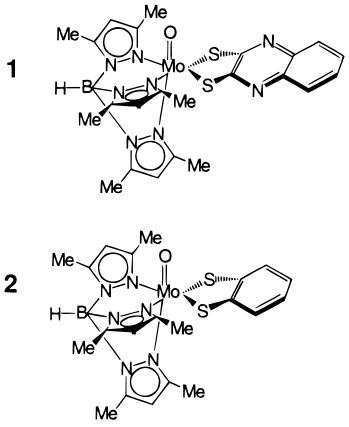


Figure 1. Two-electron oxidation of the native pyranopterin (a) must be accompanied by pyran ring opening (b) in order to attain a fully oxidized pterin (c) with an unsaturated pyrazine ring. Ring opening of the pyranopterin cofactor represents an induced internal redox reaction, whereby a two-electron oxidation of the pterin ring is coupled to a two-electron reduction of the pyran ring to an open-chain primary alcohol. Therefore, the combination of pyran ring opening and two-electron oxidation by ferricyanide represents a four-electron oxidation of the pterin to the fully oxidized form.



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(8) Gardlik, S.; Rajagopalan, K. V. *J. Biol. Chem.* **1991**, *266*, 4889−4895. There is no evidence for the S_{cys} being affected by ferricyanide in inhibited enzyme.
(9) Abbreviations: L = hydrotris(3,5-dimethyl-1-pyrazolyl)borate; (bdt) = 1,2-benzenedithiolate; (qdt) = 2,3-quinoxalinedithiolate.
(10) Inscore, F. E.; McNaughton, R. L.; Westcott, B.; Helton, M. E.; Jones, R. M.; Enemark, J. H.; Kirk, M. L. *Inorg. Chem.* **1999**, *38*, 1401−1410.
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(12) LMoO(qdt) is a first-generation model for ferricyanide-inhibited sulfite oxidase, since the pyrazine ring is directly fused to the ene-1,2-dithiolate and it lacks the potential for torsional flexibility about the $C_{ene}-C_{pyrazine}$ bond. In actuality, torsional rotation is minimized since the pyranopterin is buried in the protein and found to be extensively hydrogen bonded to a number of main and side chain atoms of protein amino acid residues.
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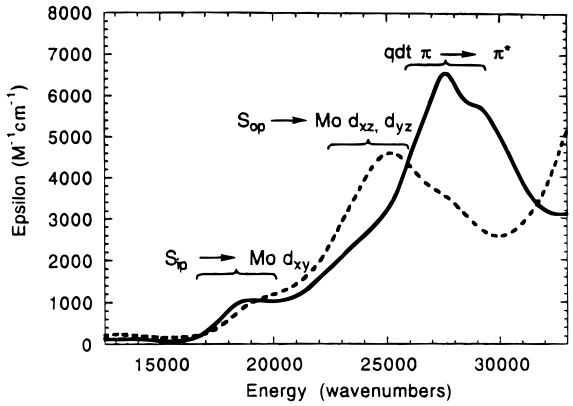

Figure 2. Room temperature electronic absorption spectra of LMoO(qdt) (solid line) and LMoO(bdt) (dashed line) in 1,2-dichloroethane. Note the large reduction in the intensity of the dithiolate $S_{op}\rightarrow$Mo $d_{xz,yz}$ transitions for **1** relative to **2**.

implying a near isoenergetic manifold of excited states for these two compounds. We have recently undertaken an extensive electronic structure study of **2**, making detailed spectral assignments of all the S→Mo CT bands which occur below ~26 000 cm^{-1},[10] and the MCD correspondence allows for transfer of these assignments to **1**. A comparison of the electronic absorption spectra for **1** and **2** is presented in Figure 2, along with the relevant band assignments.[14]

Compound **1** displays intense absorbances at 27 575 and 29 100 cm^{-1} which correspond very well to the 26 300 and 27 650 cm^{-1} bands in the ferricyanide-treated molybdenum fragment of sulfite oxidase, in addition to the lower energy S→Mo CT bands.[8] Fully oxidized pterins are known to possess absorption maxima in this region which originate from $\pi\rightarrow\pi^*$ transitions localized primarily on the oxidized pyrazine ring of the pterin.[8] Also, it is readily apparent from Figure 2 that the intensity of the dithiolate $S_{op}\rightarrow$ Mo $d_{xz,yz}$ transitions for **1** are roughly half those observed for **2**.[15] The reduction in CT intensity reflects the considerably weaker S_{op} donor ability of the (qdt) ligand relative to (bdt). This reduced charge donation results in a Mo center for **1** which is more electropositive (increased Z_{eff}) relative to that in **2**. An increase in Z_{eff} results in greater energy stabilization of the Mo d orbitals for **1**. This effect is also reflected in the He(I) PES spectrum of **1**,[16] where the first-ionization from the Mo d_{xy} redox orbital is ~0.75 eV greater than that for the related LMoO(tdt).[17] Therefore, the energetic stabilization of the Mo d_{xy} orbital in **1**, which results from the compromised S_{op} donor ability of the (qdt) ligand, is the dominant contributor to the observed +220 mV positive shift in the reduction potential relative to **2**.[18]

The present model study has direct relevance with respect to the origin of ferricyanide-induced electron transfer inhibition between the Mo(IV) site in sulfite oxidase and oxidized cytochrome *c*. There are two mechanisms, one based on the electronic coupling matrix element $(H_{AB})^2$ and the other on thermodynamic considerations ($\Delta G°$), which can affect the ET regeneration rate (k_{et}) and inhibit the reoxidation of Mo(IV) in ferricyanide-treated sulfite oxidase (eq 1). The first, $(H_{AB})^2$, results from a reduction

$$k_{et} = \sqrt{\frac{4\pi^3}{h^2\lambda kT}}(H_{AB})^2 \exp\left(\frac{-(\lambda + \Delta G°)^2}{4\lambda kT}\right) \qquad (1)$$

in the covalency of the $S_{dithiolate}$−Mo d_{xy} bonding interaction, with a concomitant reduction in the electron transfer rate.[19,20] We have shown that $S_{dithiolate}$−Mo d_{xy} bonding in LMoO(dithiolate) compounds is dominated by *in-plane* S_{ip}−Mo d_{xy} interactions.[10] However, it is readily apparent from the absorption spectra that the intensity of the dithiolate $S_{ip}\rightarrow$Mo d_{xy} transition is very similar for **1** and **2**. This is a bonding → antibonding transition, the intensity of which probes the dominant covalency contributions between the Mo d_{xy} redox orbital and the dithiolate S_{ip} orbitals.[15] Furthermore, we have provided evidence that this bonding interaction is likely to provide a superexchange pathway for electron transfer regeneration in pyranopterin molybdenum enzymes.[10] Obviously, the presence of an oxidized pyrazine ring associated with the dithiolate chelate in **1** does not attenuate the intensity of this band, indicating exceptional electronic communication between the Mo d_{xy} and dithiolate S_{ip} orbitals of **1** and **2**. The second inhibition mechanism ($\Delta G°$) results from an increase in the valence ionization energy of the Mo d_{xy} orbital, and a concomitant stabilization of Mo(IV) to such a degree that electron transfer between the reduced Mo site and oxidized cytochrome *c* would be thermodynamically uphill. This is exactly what is observed in **1**, where the potential is shifted +220 mV relative to **2**. Thus, the presence of an oxidized pyrazine ring results in an electron-withdrawing effect on the S_{op} donor orbitals of the dithiolate chelate. This causes the dithiolate sulfur atoms to become poorer donors to Mo and increases the effective nuclear charge on the metal, greatly increasing the reduction potential and severely retarding the rate (k_{et}) of ET regeneration following oxygen atom transfer.

In conclusion, we have developed a first-generation model for ferricyanide-inhibited sulfite oxidase which incorporates a pyrazine ring associated with a dithiolate chelate. The spectroscopic studies presented here provide an impressive example of orbital control over reduction potentials. This provides evidence that thermodynamic stabilization of Mo(IV), and not disruption of S_{ip}− Mo d_{xy} covalency contributions to the electron transfer regeneration pathway, is responsible for the severe reduction of sulfite: cytochrome *c* activity in ferricyanide-treated enzyme. We suggest that the inhibition results from the complete oxidation of the pyrazine portion of the pyranopterin, which causes a reduction in the donor ability of the ene-1,2-dithiolate sulfurs by an inductive effect. Furthermore, the stabilization of Mo(IV) in inhibited enzyme is postulated to arise from *anisotropic* covalency contributions involving only the S_{op} orbitals of the coordinated dithiolate to the reduction potential of the site.

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Supporting Information Available: Figure 1. Sulfite oxidase catalytic cycle involving oxygen atom and coupled electron−proton transfer. Figure 2. 5 K, 7 T MCD spectra of LMoO(qdt) (solid line) and LMoO(bdt) (dashed line) in poly(dimethylsiloxane).

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(14) S_{ip} and S_{op} refer to predominantly sulfur containing molecular orbitals of a dithiolate chelate oriented in the plane and perpendicular to the plane of the dithiolate chelate, respectively.

(15) The intensity (*I*) of a charge transfer transition is proportional to the square of the overlap integral $\langle\varphi_M|\varphi_L\rangle^2$. See, for example: Solomon, E. I. *Comments Inorg. Chem.* **1984**, *3*, 225−320.

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(17) The abbreviation (tdt) refers to the ligand 3,4-toluenedithiolate. Due to the electronic and spectral similarity of LMoO(tdt) and **2** (see ref 10), the binding energy for the Mo d_{xy} electron is anticipated to be equivalent for both compounds.

(18) Cyclic voltammetric measurements were performed on 1,2-dichloroethane solutions of **1** and **2** over a potential range of +2 to −2 V. Potentials are reported vs the ferrocenium/ferrocene couple. LMoO(qdt) (−620 mV) and LMoO(bdt) (−840 mV).

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