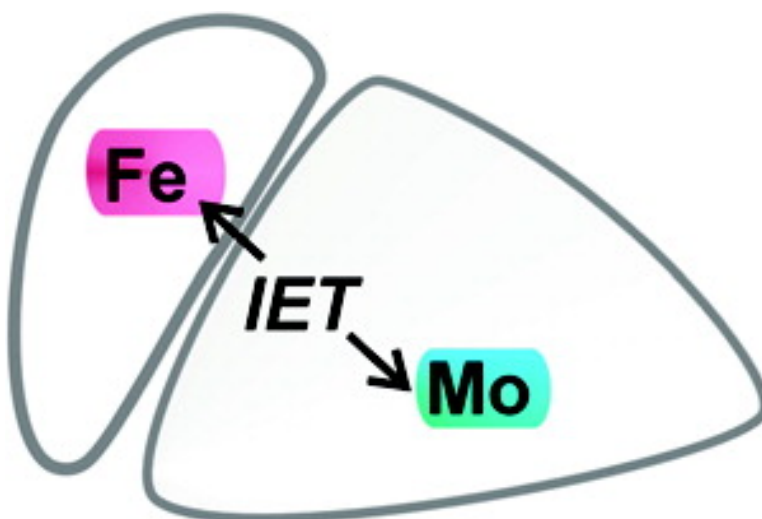Communication

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# Intramolecular Electron Transfer in a Bacterial Sulfite Dehydrogenase

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The sulfite-oxidizing molybdenum-containing enzymes can be classified into two types, sulfite oxidases (SO, found in animals and plants, EC 1.8.3.1), and sulfite dehydrogenases (SDH, found in bacteria, EC 1.8.2.1), on the basis of their ability to transfer electrons to molecular oxygen.[1,2] Amino acid sequence analyses indicate that the molybdenum cofactor binding domains are related in sulfite-oxidizing enzymes from all three sources, while the overall structure and cofactor content of the enzymes differ significantly.[3,4] SO from *Arabidopsis thaliana* contains no redox-active center other than the Mo site,[4] whereas both animal SO and bacterial SDH possess heme centers in addition to the Mo site. In the homodimeric chicken SO, each subunit contains a Mo domain and a $b_5$-type heme domain that are linked by a flexible peptide loop (Figure 1A).[5] In contrast, *Starkeya novella* SDH has a novel $\alpha\beta$-heterodimeric structure in which the Mo cofactor and the *c*-type heme are located on different subunits (Figure 1B) and the heme subunit is not related to that found in chicken SO.[3] In both Mo/heme enzymes, intramolecular electron transfer (IET) between the Mo and Fe centers is fundamental to the function of the enzymes. Comprehensive laser flash photolysis studies on chicken and human SO have shown that the first-order IET rate constants between the reduced heme (Fe$^{II}$) and oxidized molybdenum (Mo$^{VI}$) centers depend significantly on the experimental conditions (solution viscosity,[6,7] sulfate concentrations,[8,9] and pH[8,10]). To explain the remarkable dependency of IET upon viscosity, it has been proposed that chicken SO may adopt an alternative conformation to that seen in the crystal structure.[6] This would most likely be driven by electrostatic interactions between the two domains,[11] thereby facilitating rapid IET by having a much shorter heme-to-Mo distance (Figure 1A).[6] The inhibitory effect of sulfate ions on IET was considered as evidence for sulfate binding close to the Mo center,[9] which decreases the nearby positive surface charge, retarding heme docking and disfavoring fast IET.[8] Very recently, electrochemical studies also have indicated that the motion of the heme domain is a limitation for chicken SO activity,[12] and the conformational flexibility of chicken SO has been further supported by a recent pulsed ELDOR study.[13] Hoffman and co-workers have recently presented an elegant study of electron transfer in protein−protein complexes that have multiple conformations, only some of which are ET competent.[14]

In contrast, as noted above, the heterodimeric bacterial SDH represents a distinctly different type of sulfite-oxidizing enzyme in which a fairly rigid positioning of the subunits/redox centers relative to one another is necessary to maintain the enzyme's integrity.[3] IET processes in this recently described SDH have never been studied before and should be expected to differ markedly from those in chicken SO. In this study, we used laser flash photolysis under conditions analogous to those used for animal SO to
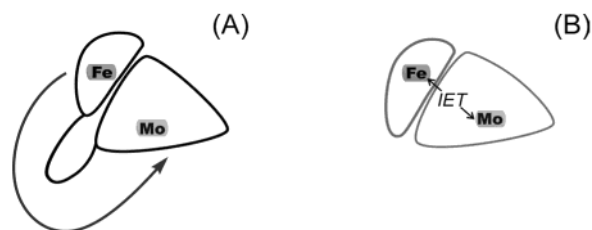


**Figure 1.** Schematic diagrams of (A) chicken SO in which the two domains are linked by a flexible peptide loop that allows conformational change prior to IET (only one subunit is shown for clarity) and (B) bacterial SDH with no linker between the Mo and heme subunits allowing direct IET.

investigate the nature of the IET processes in bacterial SDH. An additional goal was to provide further insights into the role of conformational flexibility and protein docking in the remarkable influence of sulfate anions and solution viscosity on IET in animal SO.

SDH was expressed and purified as described previously.[3,15] Small aliquots of a concentrated recombinant SDH solution (approximately 1 mM) were injected into an anaerobic cuvette containing 5-deazariboflavin (dRF) and 0.5 mM freshly prepared semicarbazide in 20 mM Bis-Tris buffer, pH 6.0. The basic photochemical process by which 5-deazariboflavin semiquinone (dRFH•) is generated and used to reduce redox-active proteins has been extensively described.[16] Using dRFH• radicals at pH 6 in the absence of sulfate anions, an initial rapid second-order reduction of the heme center[17] occurs ($k = 2 \times 10^8$ M$^{-1}$ s$^{-1}$, which is similar to the reduction rate constants for mitochondrial cytchrome *c*[18]). This is followed by a slow heme reoxidation process with an observed rate constant of 120 s$^{-1}$ (Figure 2). Note that the reoxidation process is protein concentration independent, implying that it is due to a first-order IET process from Fe$^{II}$ to Mo$^{VI}$, a process also observed in studies on animal SO,[6−11] and consistent with the evidence showing that SDH is a very tightly bound heterodimer.[3] For chicken SO, under comparable conditions, the IET rate constant is >1000 s$^{-1}$.[8,10] The difference in the magnitude of IET rate constants in chicken SO and bacterial SDH may be due to different driving forces (i.e., relative heme/Mo redox potentials) and possibly also to a different distance between the heme and molybdenum centers during the IET. The heme reoxidation is not complete, indicating that an equilibrium between Mo$^{VI}$Fe$^{II}$ and Mo$^{V}$Fe$^{III}$ (see below) is established (the equilibrium constant is designated as $K_{eq}$ = $k_f/k_r$, and is equal to $b/a$ in Figure 2; cf. Table 1 in Supporting Information), a process similar to that seen in chicken and human SO. Note that the IET rate constant is the sum of the forward electron transfer ($k_f$) and reverse electron transfer ($k_r$) rate constants.[8]

$$\text{Mo}^{VI}\text{Fe}^{II} \underset{k_r}{\overset{k_f}{\rightleftharpoons}} \text{Mo}^{V}\text{Fe}^{III}$$
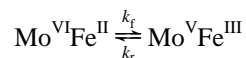
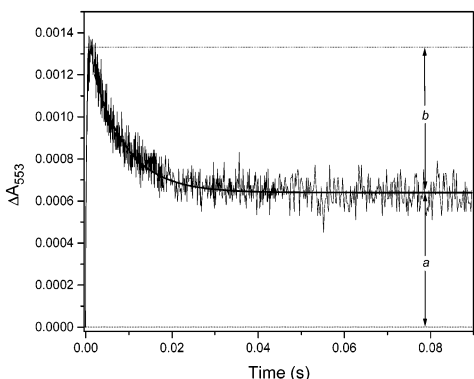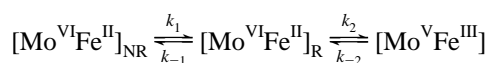Thus $k_f$ and $k_r$ in SDH at pH 6.0 can be calculated to be 66 s$^{-1}$ and

**Figure 2.** Transient kinetic trace obtained at 553 nm upon photoexcitation of a solution containing 16 $\mu$M SDH, ~90 $\mu$M dRF, and 0.5 mM semicarbazide hydrochloride in 20 mM Bis-Tris buffer (pH adjusted to 7.0 by acetic acid). The initial rise is due to heme reduction; this is followed by an absorbance decrease due to IET between the heme and molybdenum cofactors. The solid line indicates a single-exponential fit to the IET phase; $K_{eq} = b/a$.

54 s$^{-1}$, respectively. The slow absorbance decay due to IET was not observed at pH 7.4, indicating that $K_{eq}$ for IET in SDH is pH dependent, consistent with the previously observed pH dependence of the redox potential of Mo$^{VI}$/Mo$^{V}$.[1] Below pH 7.0, this potential becomes markedly more positive than that of the heme $c$ group, thereby facilitating IET in the forward reaction from Fe$^{II}$ to the Mo$^{VI}$ center ($k_f$), i.e., $K_{eq}$ becomes larger. The $k_{cat}$ of SDH is 50 s$^{-1}$ at pH 6.0,[19] indicating that the rate-limiting step in the catalytic mechanism is not the IET and that the enzyme still remains functional at this pH.

The SDH IET rate constant in 18% (w/w) sucrose is (within experimental error) the same as that in the absence of sucrose, and so are the equilibrium constants for IET (cf. Table 1 in Supporting Information). In contrast, for chicken and human SO, a substantial effect of solution viscosity on IET has been observed and has been explained by the following mechanism.[6]

$$[\text{Mo}^{VI}\text{Fe}^{II}]_{NR} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [\text{Mo}^{VI}\text{Fe}^{II}]_R \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} [\text{Mo}^{V}\text{Fe}^{III}]$$

This scheme proposes the existence of two conformationally different precursors prior to IET, designated NR (for electron-transfer nonreactive) and R (for electron-transfer reactive), respectively, whose interconversion (occurring via the two first-order rate constants $k_1$ and $k_{-1}$) is retarded by high solution viscosity.[6] The absence of a viscosity effect on IET in the bacterial SDH strongly suggests that IET in this protein (occurring via the two first-order rate constants $k_2$ and $k_{-2}$) occurs directly through the protein medium (Figure 1B) and does not involve a significant conformational change, consistent with the presence of a tightly bound cytochrome subunit. It is reasonable to surmise that SDH has a favorable Mo···Fe distance and/or intervening amino acid arrangement that allows IET to proceed in this manner. To confirm this, the crystal structure of the SDH will have to be determined.

The SDH IET rate constant and its equilibrium constant also remain unchanged in the presence of 55 mM sulfate (cf. Table 1 in Supporting Information). As has previously been shown,[7,9] IET rates in human and chicken SO are strongly inhibited by sulfate binding. It has been proposed that sulfate binding near the Mo center in chicken SO decreases the positive charge on the surface of the Mo domain, thereby retarding the docking of the heme domain to the Mo domain, thus inhibiting IET.[8] As noted above, due to SDH's distinctly different structure, IET in the enzyme from *S. novella* takes place via a very different mechanism (Figure 1B), which does not involve significant protein motion/docking, and therefore sulfate cannot inhibit IET in this protein by masking surface charges necessary for the repositioning of the protein domains. Sulfate binding does, however, inhibit the forward reaction of the enzyme with a $K_i$ of 8 mM,[3] an effect that is likely caused by a competition of sulfate and sulfite for the substrate-binding molybdenum site.

In summary, IET between the Mo and Fe centers in the bacterial SDH was investigated here for the first time. In contrast to animal SO, the rate constants of IET in SDH are not affected by viscosity or the presence of sulfate, indicating that IET in SDH proceeds directly within the protein medium and does not involve substantial movement of the two subunits relative to each other. The present results also provide direct evidence that the substantial influence of sulfate anions and solution viscosity on IET in animal SO$^{6-10}$ is due to the retardation of interdomain docking in these multidomain SO and is not an inherent property of all sulfite-oxidizing molybdoenzymes.

**Supporting Information Available:** Figures S1–S4, flash-induced and steady-state difference spectra of SDH, transient kinetic traces obtained at 553 nm upon photoexcitation of SDH solution containing 18% (w/w) sucrose or 55 mM sodium sulfate; Table 1, IET rate constants ($k_{et}$) and equilibrium constants ($K_{eq}$) of SDH under different experimental conditions (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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