Molecular Basis of Intramolecular Electron Transfer in Sulfite-oxidizing Enzymes Is Revealed by High Resolution Structure of a Heterodimeric Complex of the Catalytic Molybdopterin Subunit and a c-Type Cytochrome Subunit*

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Sulfite-oxidizing molybdoenzymes convert the highly reactive and therefore toxic sulfite to sulfate and have been identified in insects, animals, plants, and bacteria. Although the well studied enzymes from higher animals serve to detoxify sulfite that arises from the catabolism of sulfur-containing amino acids, the bacterial enzymes have a central role in converting sulfite formed during dissimilatory oxidation of reduced sulfur compounds. Here we describe the structure of the Starkeya novella sulfite dehydrogenase, a heterodimeric complex of the catalytic molybdopterin subunit and a c-type cytochrome subunit, that reveals the molecular mechanism of intramolecular electron transfer in sulfite-oxidizing enzymes. The close approach of the two redox centers in the protein complex (Mo–Fe distance 16.6 Å) allows for rapid electron transfer via tunnelling or aided by the protein environment. The high resolution structure of the complex has allowed the identification of potential through-bond pathways for electron transfer including a direct link via Arg-55A and/or an aromatic-mediated pathway. A potential site of electron transfer to an external acceptor cytochrome c was also identified on the SorB subunit on the opposite side to the interaction with the catalytic SorA subunit.

Sulfite oxidases (SO(s))† and nitrate reductases are members of a subclass of molybdopterin enzymes characterized by the presence of a MoO2 metal center. The enzymes typically contain a molybdopterin cofactor (Moco) implicated in substrate binding and oxidation and a heme cofactor for molybdenum reoxidation. Three types of sulfite-oxidizing enzymes have been characterized so far, namely the homodimeric, heme b and molybdenum-containing enzymes from higher animals and birds (1, 2), the homodimeric, molybdenum-containing enzymes from plants (3) and a heterodimeric, heme c and molybdenum-containing bacterial enzyme (4) that cannot transfer electrons to molecular oxygen and is therefore classified as a sulfite dehydrogenase. Of these enzymes, the sulfite oxidases from higher animals and birds have been studied in great detail, as in humans a deficiency in SO activity is linked to severe developmental retardation and early death of the affected individuals (2, 5). The crystal structures of two homodimeric eukaryotic sulfite oxidases, CSO from chicken liver (2) and PSO from Arabidopsis thaliana (6), have been determined and have provided structural details of the molybdenum center and substrate binding pocket. During catalysis two electrons are transferred from the substrate, sulfite, to the fully oxidized Mo(VI) form of the active site, which is then reduced to the Mo(IV) state. The two electrons are then transferred sequentially from the molybdenum to an electron acceptor (PSO) or to the heme cofactor, which can be present either as a domain (e.g. CSO, human sulfite oxidase (HSO)) or subunit (e.g. bacterial sulfite dehydrogenase) of the enzyme. Using the structural information and the results from site-directed mutagenesis studies on HSO (7–9) it has been possible to assign functions, especially in substrate binding, to various residues that are conserved in the vicinity of the active site. Intramolecular electron transfer (IET) between Moco and the heme is fundamental to the function of the mammalian and bacterial enzymes; however, the electron transfer pathway between the molybdenum and the heme cofactor has not yet been determined. One reason for this is the way in which the heme domain is attached to the rest of the protein in CSO and HSO, namely as a separate domain that is connected to the Moco/ dimerization domains of the protein by a flexible linker that allows for extensive changes in its position relative to the other two domains of the protein. In fact, in the published CSO structure the position of the heme domain is removed from the active site (2, 10) with a distance of 32 Å between the two metal centers. This distance cannot be expected to allow for efficient electron transfer, and this enzyme structure has been presumed to be in a “redox-inactive” state. The viscosity of the medium has a marked effect on IET in CSO, which suggests that domain movement is essential for catalysis in this protein (11). At present, it is unknown how the heme domain of the vertebrate enzymes is attached to the Moco domain during transfer of electrons or how this domain interacts with its external electron acceptor, cytochrome c. Here we present a description of the crystal structures of the reduced state of the heterodimeric sulfite dehydrogenase (SorAB) from the soil bacterium Starkeya novella (4). This structure is the first for a multisubunit sulfite-oxidizing enzyme and allows direct insights into the electron transfer pathway between the molyb-
Electron Transfer in Sulfite-oxidizing Proteins

Purification and Crystallization—Recombinant sulfite dehydrogenase was purified and crystallized as described previously (12). To generate fully oxidized protein samples, purified protein was mixed with excess cytochrome c (horse heart, C-7752, Sigma), incubated on ice for 1 h and then subjected to size-exclusion chromatography (Superdex 75 6/60 prep grade, GE Healthcare) to remove the horse heart cytochrome.

Data Collection—Data were collected on beamlines at the Advanced Light Source, Berkeley and at the SRS, Daresbury Laboratory. Although three-wavelength multiwavelength anomalous diffraction data were collected for the sodium bromide-soaked crystal, only one peak wavelength was used in the structure determination. All data were processed using the program Mosflm (13) and subsequently scaled together, reduced, and truncated using the CCP4 Suite (14). Data collection and processing statistics are shown in Table I.

Structure Determination—The crystal structure has been determined by a combination of molecular replacement and heavy atom substitution. The search model for molecular replacement was derived from the structure of CSO (PDB entry 1SOX). The best molecular replacement solution, determined using the program MOLREP (15, 16), was obtained using the Moco domain of CSO (residues 106–308) modified by truncation of protein side chains to the atoms common between the two proteins. The position of the dimerization domain (residues 316–453) was subsequently determined using the program FFFEAR (17). Several low occupancy heavy atom sites in the derivatives could be determined from the positions of consistent peaks in heavy atom difference and anomalous difference Fourier maps calculated using the molecular replacement phases. The position of the cytochrome iron was also determined from anomalous difference Fourier maps using data collected on the high energy side of the iron absorption edge. The combined molecular replacement and multiple isomorphous replacement phases were used with the native 1.8 Å resolution data as the starting point for automated model building using ARP/wARP (18) generating a model comprising 460 residues. The remaining residues and the cofactors were built manually using the program O (19). Refinement was completed using the program REFMAC (20), and refinement statistics are given in Table I. The native data in Table I were used for structure determination and refinement. The organic part of Moco is very well defined; however, the molybdenum is not fully occupied in this crystal, and refinement with an occupancy of 0.6 gave a reasonable model.

The final models consist of residues 1–373 of the SorA subunit, residues 1–81 of the SorB subunit, one Moco, one c-type heme, a sulfate ion, and water molecules. Three disulfide bonds were identified at positions Pro-31A, Pro-114A, and Pro-200A of the SorA chain, and a disulfide bond is present between Cys-29A and Cys-32A. Subsequent structures of cytochrome-oxidized and sulfite-reduced enzyme were made to determine structures of SorAB in the oxidized and reduced states (Table I). The structures of cytochrome-oxidized and sulfite-reduced enzyme showed no significant differences, and both structures appear compatible with the reduced state of Moco, possibly because of photoreduction by the x-rays during data collection. Details of the structure described in this paper are from the model refined against the sulfite-reduced data in Table I.

SorAB is a complex of two subunits: SorA, a Moco binding subunit, and SorB, a cytochrome subunit (Fig. 2) (4). SorA, the catalytic subunit, contains two domains that resemble the homologous domains of CSO and PSO (2, 6). As seen in those structures, domain 1 (residues 1–254) has a mixed a/b-fold and is responsible for binding Moco (Fig. 3), whereas domain 2 (residues 255–373) has an antiparallel β-barrel structure with the same topology as an immunoglobulin fold. Interestingly, one long loop of domain 2 that deviates from the core immunoglobulin fold stretches down into the Moco domain and contributes to the surface of the substrate binding channel (Fig. 1B). Domain 2 is responsible for formation of the dimerization interface in the homodimeric eukaryotic sulfite oxidases but has no immediately apparent function in SorAB.

The SorB subunit has a c-type cytochrome fold (22) and is predominantly α-helical in character with the exception of the N-terminal residues, which extend away from the core of the subunit and wrap around the Moco domain. SorB contains the typical heme binding motif of a type I cytochrome c, namely the consensus sequence CXXCH close to the N terminus, and binds a single c-type heme with histidine-methionine axial coordination. The heme group is covalently attached by thioether bonds to the two conserved cysteine residues Cys-29B and Cys-32B.
A search of the RSCB protein data bank (www.rscb.org/pdb) found that SorB is most similar to the N-terminal subdomain of chain A of quinohemoprotein amine dehydrogenase from Paracoccus denitrificans (PDB entry 1jju) (23), which shares 28% sequence identity. This subdomain is half of a diheme cytochrome domain and 58 Cα atoms can be structurally aligned with an r.m.s.d. of 1.2 Å. One common feature of these cytochromes is the absence of long loops between helices 1 and 2 and helices 3 and 4, which are present in the majority of single heme bacterial c-type cytochromes and shield the heme cofactor from solvent. Thus both propionate groups of the heme moiety of SorB are exposed to water molecules, which are present at the SorA-SorB interface.

Pyrrrole ring C of the heme in the isolated cytochrome would also be exposed to solvent, however, in the SorAB complex, loop 160–167 provided by the Moco domain interacts with the cytochrome at this edge of the heme. With the exception of closely related sulfite dehydrogenase cytochromes, SorB has low homology and poor structural alignment with other monodomain c-type cytochromes.

**Interactions between SorA and SorB**—The SorAB complex can be purified from *S. novella* and is stable and active, suggesting a subunit interaction that is both specific and of relatively high affinity. This is the first example of a sulfite-oxidizing protein where the cytochrome is present as a separate subunit and in a position to carry out physiological IET, and

### Table I

| Data collection and refinement statistics |
|------------------------------------------|
| **Data collection**                      |
| Crystal | Native<sup>a</sup> | Iron<sup>b</sup> | Xenon | Bromine | Cytochrome-oxidized<sup>c</sup> | Sulfite-reduced<sup>h</sup> |
| Beamline | ALS | ALS | SRS 14.1 | ALS | SRS 10.1 | SRS 10.1 |
| Wavelength (Å) | 1.0 | 1.737 | 1.488 | 0.9191 | 1.074 | 1.074 |
| Resolution range (Å) | 67–1.8 | 56–2.5 | 67–2.5 | 67–2.5 | 50–1.7 | 36–1.9 |
| Unique reflections | 45440 | 17022 | 18307 | 18238 | 56105 | 40189 |
| Completeness (%) | 95.6 (77.5) | 93.6 (93.5) | 99.4 (96.0) | 100 (100) | 98.3 (90.4) | 97.9 (97.7) |
|Multiplicity<sup>f</sup> | 5.7 (3.0) | 3.4 (3.1) | 8.5 (7.0) | 7.1 (7.1) | 4.7 (3.8) | 4.2 (3.7) |
| I/σ(I) | 9.1 (3.5) | 6.3 (5.2) | 8.8 (4.4) | 7.3 (4.1) | 14.1 (2.1) | 12.5 (2.8) |
| R<sub>merge</sub> (%)<sup>d</sup> | 6.5 (19.3) | 7.6 (11.9) | 6.2 (11.9) | 8.5 (16.8) | 10.3 (53.5) | 11.0 (44.5) |
| **Refinement statistics** |
| Resolution range (Å) | 20–1.8 | 20–1.7 | 20–1.9 |
| R<sub>cryst</sub> (%)<sup>e</sup> | 13.6 | 16.7 | 16.6 |
| R<sub>free</sub> (%) | 16.9 | 19.7 | 20.9 |
| r.m.s.d. from ideal geometry | 0.010 | 0.014 | 0.014 |
| Bond lengths (Å) | 1.3 | 1.5 | 1.5 |
| Number of water molecules | 583 | 474 | 360 |
| Average B-factors (Å<sup>2</sup>) | SorA atoms | 12.5 | 23.6 | 15.7 |
| | SorB atoms | 14.6 | 25.6 | 17.5 |
| | Water atoms | 27.2 | 37.3 | 24.2 |

<sup>a</sup>Protein was reduced by the presence of 2 mM sodium sulfite in the crystallization drops and cryoprotectant.

<sup>b</sup>Data were collected on the high energy side of the iron absorption edge.

<sup>c</sup>Values in parentheses refer to the highest resolution shell.

<sup>d</sup>R<sub>merge</sub> = ΣΣΣ[I<sub>hkl</sub> - I<sub>i</sub>]/ΣΣΣ[I<sub>i</sub>], where I<sub>hkl</sub> and I<sub>i</sub> are the observed and calculated structure factor amplitudes.

<sup>e</sup>R<sub>cryst</sub> was calculated with 5% of the data that had been excluded from refinement.

<sup>f</sup>Protein was oxidized with cytochrome c prior to crystallization.

<sup>g</sup>Crystals were reduced by the addition of 1 mM sodium sulfite 1 min prior to cryocooling.

**Fig. 2.** A, ribbon diagram of SorAB showing secondary structure elements. The Moco and heme cofactors are drawn as stick models with the metal centers as green spheres. B and C, surface rendering of front and back faces of SorAB. SorA subunit, comprising the Moco and dimerization domains colored blue and violet, respectively and SorB the cytochrome c subunit is colored green. Waters in the active site are shown as red spheres. The heme is colored yellow and is exposed to solvent on both the front and back faces.
therefore the nature of the subunit interface in the complex is of some interest.

The cytochrome interacts only with the Moco domain of SorA, forming an interface composed of two distinct contact regions. One region involves interactions between the core of the cytochrome and SorA, whereas the second contact region is provided by the N-terminal arm of the cytochrome (residues 1–10), which extends 25 Å along the surface of SorA (Fig. 2, B and C) and is reminiscent of the NapB subunit of the heterodimeric periplasmic nitrate reductase from *Rhodobacter sphaeroides* (24). The total solvent-accessible surface area buried on complex formation is 2800 Å², with approximately two-thirds contributed by region 1 and one-third by region 2. Such extensive protein-protein interactions are commensurate with a permanent heterodimeric complex (25).

A total of 30 direct hydrogen bonds, including two salt bridges (Arg-30A–Glu-10B and Arg-55A–propionate-6B), are formed at the subunit interface, a large number compared with an average of 10 hydrogen bonds/interface in a recent study of 75 protein-protein complexes (26). One of the salt bridges, between Arg-30A and Glu-10B, is at the junction between the arm and core of the cytochrome, and both residues are conserved in SorAB-related sequences. The interface between the core of the cytochrome and SorA covers one-half of an elongated cleft leading to the substrate binding site of SorA. However, the cytochrome does not make close contact with SorA in this cleft, thus, solvent and substrate are able to access the channel between the two subunits on one side of the interface (Fig. 2B).

### FIG. 3. Moco domain.

Primary sequence alignment and secondary structure of representatives of mammalian (SUOXCHI), plant (ATHPSO1), and bacterial (SNOsorA) sulfite-oxidizing enzymes, plus representatives of the assimilatory nitrate reductases (NIAATH) and the SoxCD (PDESoxC) sulfur dehydrogenases also belonging to the sulfite oxidase protein family. Active site residues are highlighted (green), residues hydrogen bonding to the molybdenum cofactor are shown in transparent boxes with black outline, the conserved active site cysteine is shown in a gray box, cysteines involved in disulfide bonds are shown in bold, and residues in loops that vary between the SorAB, CSO, and PSO models are shown with a black line above.
complementary electrostatic surfaces at the interface of the SorA and SorB subunits (Fig. 4).

Comparison of the Overall Fold of SorAB, CSO, and PSO—
The domains of subunit SorA have the same fold as the Moco and dimerization domains of CSO and PSO, and can be superimposed on these domains with an average r.m.s.d. of 1.34 and 1.38 Å for CSO and PSO, respectively (Fig. 5). If the Moco and dimerization domains are each superimposed independently the fit is improved, indicating a small shift in the relative positions of these domains that has not been observed in the CSO or PSO structures (Table II).

Although the Cu atoms in the secondary structural elements of all three sulfite-oxidizing enzymes align well, there are large differences in four surface loops (Fig. 3, Fig. 5A) and the position of the respective N termini. Loops 1 and 2, residues 145–150 and 315–318 (SorA numbering), exhibit large differences in all three proteins and are not involved in any known interactions. A third loop, residues 159–167, is also different in all three proteins, and in SorAB this loop and the N-terminal residues 9–19 form interactions with the cytochrome subunit. Of particular interest are the structural differences observed for the fourth loop, residues 239–254, which adopts the same conformation in CSO and PSO and forms interactions at the dimer interface of these enzymes. In the heterodimeric SorAB this loop contains an unconserved disulfide bond between Cys-243A and Cys-245A, and the altered conformation causes steric hindrance that likely prevents the formation of a similar dimer interface in SorAB. The question arises as to what the function of this domain and the dimerization seen in all other SOs so far is, as it is obviously not necessary for catalytic function in SorAB, which is a highly active and specific sulfite dehydrogenase.

A major difference observed when SorAB is superimposed on CSO is the position of the cytochrome subunit relative to the Moco domain and the active site (Fig. 5B). Although in the CSO structure the heme b domain is located 32 Å from the molybdenum site, in our model, the cytochrome subunit interacts strongly with the Moco domain adjacent to the active site and forms one side of the channel leading to the substrate binding site. The relative position of the SorA and SorB subunits allows a very close approach of the two cofactors. The Mo–Fe distance is 16.6 Å, and the closest approach between the molybdenum and the edge of the heme cofactor is 8.5 Å. The short Moco–heme distance and the presence of strong electrostatic interactions between the two subunits clearly suggest that they are in the physiological positions for catalysis (27).

A comparison of the cytochromes of CSO and SorAB shows that despite the different topology, they have similar features including relatively exposed heme groups (Fig. 5, C and D). In fact, the position of SorB is similar to a suggested physiological position for the CSO heme b domain (28). Attempts to fit the cytochrome domain of CSO into that position by superposition of the CSO heme moiety on the SorAB heme generated only minor steric clashes. Based on these results it seems likely that the b-type cytochrome of CSO docks close to the CSO active site and that the exposed heme propionate is involved in a heme–Arg interaction similar to that seen for SorA-SorB.

Molybdopterin Cofactor—SorAB has a single, tricyclic molybdopterin cofactor that is tightly bound and buried in the Moco domain. The molybdopterin is not conjugated to a nucleotide moiety, which is present in most known bacterial molybdenum enzymes except those belonging to the xanthine oxidase family (29, 30). There are a total of 13 hydrogen bonds between the organic and phosphate moieties of the cofactor and the protein. With the exception of the conservative substitution of Asn-197A in SorA for His-283 of CSO, the residues with side chains involved in Moco binding are conserved with other sulfite oxidases and plant nitrate reductases (Fig. 3) (2, 6).

The molybdenum has five ligands arranged in an approximate square pyramidal geometry, with two thiolate sulfur ligands contributed from the molybdopterin, Cys-104A S–γ, an axial oxo group, and a second equatorial oxygen, which is probably a hydroxo or water molecule based on the distance to the molybdenum (Fig. 6, A and C). In their oxidized form, proteins of the sulfite oxidase family contain a dioxo-Mo(VI) center (31), and the presence of a hydroxo or water molecule is in agreement with the expected reduced state of the enzyme, because of the presence of 1 mM sulfite. The axial oxo ligand is 1.7 Å from the molybdenum and is buried, making three hydrogen bonds to the surrounding residues Ser-105A O–γ, Ser-105A N, and Thr-211A N. The hydroxo or water ligand is 2.3 Å from the molybdenum and makes hydrogen bonds with Tyr-236A OH, Arg-55A NE, and two or three water molecules in the substrate binding channel.

Substrate Binding Site—In our crystallization medium we have 2 mM sulfate, and 1 mM sulfite is added to the cryoprotectant; however, there is no clear density for either in the substrate binding site of the refined structure, which is surprising because sulfate is an inhibitor of SorAB activity. The substrate binding site was therefore identified by analogy with the CSO structure where sulfate was clearly bound close to the molybdenum (Fig. 6B). Superposition of the SorAB and CSO protein backbone and conserved residues within an 8-Å sphere of the CSO substrate molecule reveals a close fit with an r.m.s.d. of 0.70 Å for 134 matched atoms. A total of 11 residues have side chains within 6 Å of the sulfate in both SorAB and CSO, and of these 4 are conserved: Tyr-236A, Arg-55A, Arg-

![Fig. 4. Electrostatic potential map of the SorA and SorB subunits at the interface. A, SorA subunit with SorB shown as a Ca trace. B, SorB subunit with SorA shown as a Ca trace after 180° rotation compared with A.](image-url)
109A, and His-57A (SorAB numbering). Three of these residues (Tyr-236A, Arg-55A, and Arg-109A) are conserved throughout sulfite-oxidizing enzymes and nitrate reductases and form direct interactions with the sulfate in CSO. Tyr-236A and Arg-55A additionally interact directly with the hydroxo or water ligand to the molybdenum center. Site-directed mutagenesis of the equivalent of Arg-55A and Tyr-236A side chains in HSO has a significant effect on $K_M$ (sulfite) confirming a role in substrate binding (8, 32). His-57A is conserved in nearly all related proteins, with occasional conservative substitution by asparagine, reflecting its interactions with Moco rather than a role in substrate binding. Gln-33A of SorA is a conservative substitution for the more usual Asn in this position. In both SorA and CSO, the side chain of this residue makes a hydrogen bond with Arg-55A and Arg-138.

Several residues that surround the substrate binding site and that are conserved in eukaryotic sulfite-oxidizing enzymes are replaced in SorA. The majority of these non-conserved residues replace a large side chain in CSO with a smaller side chain in SorA, resulting in a more open binding site for SorA (Fig. 6, A and B). Especially intriguing for the mechanism of action is the substitution of Arg-450 (CSO) and Arg-374 (PSO) by Ala-358A in SorAB. The conformation of this Arg in eukaryotic SOs has been suggested to be important for substrate binding. Thus, this variation in the surrounding of the active site found here for SorAB is of fundamental importance for future studies of these enzymes and their mechanisms. Trp-204 of CSO also interacts directly with the substrate and is replaced by Leu-121A in SorAB. No additional direct interactions with the substrate are made in SorA to replace those lost, although Tyr-360A, found in most sulfite dehydrogenase sequences, is placed 2–3 Å from the position of the CSO Arg-450 where it could form an indirect, solvent-mediated interaction to the substrate. Arg-450 is conserved in all eukaryotic sulfite oxidases and nitrate reductases, whereas Trp-204 is conserved in eukaryotic sulfite oxidases but is replaced by a methionine in nitrate reductases. Other non-conserved residues surrounding the substrate binding site of SorA are Gly-119A and Ala-235A replacing CSO Leu-202 and Asp-321, respectively.

The wider substrate binding site of SorA accommodates a
number of water molecules (Fig. 6A). Four water molecules sit close to the sulfate position of CSO and are probably displaced on substrate binding. Three or four other water molecules fill the space left by the side chains of Arg-450 and Leu-202 of CSO, and two of these are likely to be within hydrogen-bond distance of the bound substrate. Further, water molecules are present in the channel leading to the substrate binding site, giving a total of 20–25 well defined waters between the two subunits of SorAB.

Intramolecular Electron Transfer—With the SorA-SorB interaction and the molecular environment of the redox centers defined it is possible to analyze the likely route of the intramolecular electron transfer path. The distance between the closest atoms of the two cofactors in the complex, the molybdenum atom of the molybdopterin and propionate-6 of the heme is 8.5 Å. A survey of redox proteins with known atomic structures has shown that electrons can travel up to 14 Å between redox centers through the protein medium and suggest that redox center proximity alone is sufficient to allow tunnelling of electrons at rates faster than the substrate redox reactions supported (27). Other work suggests that optimum electronic coupling pathways between the redox sites can be identified within the protein structure (33). Direct interactions between the heme moiety and SorA are mediated by propionate-6 and promote the short edge-to-edge cofactor distance (Fig. 6C), specifically, 3–4 hydrogen bonds are involved in this interaction: Arg-55A NH-1 (2.8 Å), Arg-55A NH-2 (3.3 Å), Tyr-56A O (3.5 Å), and Gln-33A NE-2 (3.6 Å). His-57A is also in a suitable position to hydrogen bond to the propionate but is more likely to be rotated and make a conserved hydrogen bond to the pterin cofactor. The distances of the guanidinium group of Arg-55A to the iron and molybdenum atoms are 11.0 and 4.0 Å, respectively, and in addition to the propionate interaction, Arg-55A forms hydrogen bonds with the substrate molecule and with the hydroxo ligand group of the molybdenum center. It is this ligand that is involved in sulfite dehydrogenase catalysis by being the target of an S-nucleophilic attack by the substrate molecule. Thus, the Arg-55A-propionate-6 interaction sits directly between the redox centers and provides a possible electronic coupling pathway.

An alternative pathway for electronic coupling between molybdenum and iron could involve a chain of aromatic residues, Phe-168A, Tyr-236A, Trp-231A, Phe-230A, and Tyr-61B, which span the space between the two redox centers with all edge-to
Electrostatic potential map of the surface of SorAB. The black arrow indicates a negative area on the surface of the cytochrome subunit at the point where the heme is exposed to solvent as shown in Fig. 2.

edge-distances of ∼4 Å (Fig. 6C). The aromatic rings of the two residues at the ends of this chain, Phe-168A and Tyr-61B, stack against the aromatic pterin cofactor and pyrrole ring D of the heme cofactor. Future mutagenesis, structural, and kinetic studies in this tightly locked system should provide considerable insight concerning electron transfer between the molybdenum and heme domains in both bacterial and vertebrate sulfite-oxidizing enzymes.

Following sulfite oxidation, SorAB needs to interact with an external electron acceptor which has been identified as a cytochrome c550, for re-oxidation of the SorB heme and thus the molybdenum center (4, 34). Two possible binding sites for an external acceptor, where the SorB heme is exposed to the surface, can be identified. One of these is in the substrate binding channel which, however, is probably too narrow to allow a close approach of the cytochrome c550. The second is on the opposite side of SorB, away from the molybdenum active site where pyrrole ring C is exposed (Figs. 2C and 7). Electrostatic calculations show that the surface surrounding this area is predominantly negatively charged, suitable for interacting with the positively charged cytochrome c550 (Fig. 7). We propose that this second position is the cytochrome c interaction site.

Sulfate ions cause strong inhibition of both catalytic activity and IET in CSO (1, 11), but in contrast, sulfite does not appear to inhibit IET in SorAB as measured by laser flash photolysis (35). It has been suggested (8, 11, 36) that the strong inhibitory effect of sulfate ions on CSO IET can be explained by sulfate bound in the substrate binding pocket close to the molybdenum center decreasing the positive electrostatic potential in this vicinity and thereby decreasing attraction for the negatively charged heme domain. The published CSO model contains two sulfate ions, one very close to the molybdenum and occupying the probable substrate binding site, and the second is 5.5 Å from Arg-138 (CSO numbering) at the top of the cleft leading to the substrate binding channel, in a negatively charged pocket created by two symmetry-related molecules in the crystal. On examination of the superimposed SorAB and CSO structures, this second sulfate is positioned ∼3 Å from the position of the heme propionate-6 acidic moiety of SorB where it is likely to interfere with the docking of the negative charge. The binding of sulfate at this second position is not possible in SorAB because of the stability of the complex and the proximity of the heme propionate-6. Thus, it is possible that binding of sulfate at the surface of the substrate binding channel contributes to the decrease in positive electrostatic potential and to sulfate inhibition of IET in SOs.

Conclusions—The structure of a stable complex of the catalytic SorA subunit with its electron transfer partner, the c-type cytochrome SorB subunit is presented here and provides new molecular insights into the mechanism of IET in sulfite-oxidizing enzymes. Although the subunit structure of the heterodimeric SorAB contrasts with the homodimeric structure of the eukaryotic SOs, enzymatic characterization has established that the enzymes exhibit similar catalytic parameters (4). From the structural data presented, it is evident that Arg-55A has a critical role in both steps of catalysis, oxidation of sulfite and subsequent transfer of electrons from Mo(IV) to the heme iron. Mutagenesis of the corresponding residue in HSO had previously identified it as involved in substrate binding and had also shown impaired electron transfer in such a mutant. Our structure clearly shows for the first time how Arg-55A is involved in electron transfer: Through its direct interaction with propionate-6 of the heme moiety, Arg-55A provides stability to the subunit interface and effectively locks the redox partners into an optimal position for electron transfer to occur. Moreover, the now apparent dual interaction of Arg-55A with the heme propionate and the catalytically active oxo/hydroxo-ligand of the molybdenum center demonstrates that it is a key residue for all aspects of catalysis in SOs and sulfite dehydrogenases. Arg-55A is also likely to play a role in the electronic coupling pathway between redox centers promoting fast IET over a metal-metal distance of 16.6 Å. Together with the evidence presented here we suggest that the eukaryotic SOs likely form a similar arginine-heme propionate interaction.

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Fig. 7. Electrostatic potential map of the surface of SorAB. The black arrow indicates a negative area on the surface of the cytochrome subunit at the point where the heme is exposed to solvent as shown in Fig. 2.

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