We have used site-directed mutagenesis to study the interactions between the molybdo-bis(molybdopterin guanine dinucleotide) cofactor (Mo-bisMGD) and the other prosthetic groups of *Escherichia coli* Me₆SO reductase (DmsABC). In redox-poised preparations, there is a significant spin-spin interaction between the reduced 

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\Delta E_{m,7} = -120 \text{ mV} \] 

[4Fe-4S] cluster of DmsB and the Mo(V) of the Mo-bisMGD of DmsA. This interaction is significantly modified in a DmsA-C38S mutant that contains a [3Fe-4S] cluster in DmsA, suggesting that the [3Fe-4S] cluster is in close juxtaposition to the vector connecting the Mo(V) and the 

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\Delta E_{m,7} = -120 \text{ mV} \] 

cluster of DmsB. In a DmsA-R77S mutant, the interaction is eliminated, indicating the importance of this residue in defining the interaction pathway. In ferricyanide-oxidized glycerol-inhibited *DmsA-C38SBC*, there is no detectable interaction between the oxidized [4Fe-4S] cluster and the Mo-bisMGD cofactor of the catalytic subunit. Except for a minor broadening of the Mo-bisMGD, except for a minor broadening of the Mo-bisMGD of DmsA.

In DmsABC, two of the [4Fe-4S] clusters of DmsB appear to be thermodynamically competent to transfer electrons from menaquinol (MQH₂, reduced midpoint potential at pH 7 

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\Delta E_{m,7} = -70 \text{ mV} \] 

Mo(V) to the Mo-bisMGD of DmsA (Eₗₐₜ values as follows: MoIV/V = -175 mV; MoVVI = -15 mV (19)).

In a DmsA-R77S mutant, the interaction is eliminated, indicating the importance of this residue in defining the interaction pathway. In ferricyanide-oxidized glycerol-inhibited *DmsA-C38SBC*, there is no detectable interaction between the oxidized [4Fe-4S] cluster and the Mo-bisMGD cofactor of the catalytic subunit.

The two remaining low potential clusters of DmsB (Eₗₐₜ values as follows: MoIV/V = -175 mV; MoVVI = -15 mV (19)), and these have Eₗₐₜ values of -50 and -120 mV, respectively (2, 6). It has been demonstrated that there is a significant conformational link between the Eₗₐₜ = -50 mV [4Fe-4S] cluster and a single menaquinol (MQH₂) binding side associated with Hia-65 of DmcA (6, 20). This was established by using the MQH₂ analog 2-n-heptyl-4-hydroxyquinoline-N-oxide to elicit an EPR line shape change on a genetically engineered [3Fe-4S] cluster in a DmsB-C102S mutant (4, 6). In this mutant, the Eₗₐₜ = -50 mV cluster is replaced by a high potential [3Fe-4S] cluster, and therefore a role for the Eₗₐₜ = -50 mV [4Fe-4S] cluster of the wild-type enzyme can be envisioned in electron transfer from MQH₂. The other cluster that appears to be thermodynamically competent to participate in catalytic electron transfer is the Eₗₐₜ = -120 mV cluster. It has been demonstrated that there is a strong spin-spin interaction between this cluster and the Eₗₐₜ = -50 mV cluster of the wild-type enzyme, consistent with both of them being part of an eight-iron [2[4Fe-4S]] ferredoxin motif (2, 6). This pair may provide a conduit for electron flow through DmsB to the Mo-bisMGD of DmsA. The two remaining low potential clusters of DmsB (Eₗₐₜ = -240 and -330 mV) may play a role in defining the overall structure of this subunit in a manner similar to that suggested for the low potential [4Fe-4S] clusters of *E. coli* nitrate reductase A (21) and fumarate reductase (22).
saturation properties of the Mo-bisMGD Mo(V) EPR spectrum are sensitive to the redox state of the $E_{m,7} = -120$ mV [4Fe-4S] cluster of DmsB (2). DmsA also contains a vestigial [4Fe-4S] cluster binding motif close to its N terminus, which, when appropriately mutagenized, can be made to bind an engineered [3Fe-4S] cluster with an $E_{m,7}$ of approximately 178 mV (23). This motif has been shown to be involved in physiological electron transfer from the [4Fe-4S] clusters of DmsB to the Mo-bisMGD cofactor of DmsA (24). This suggests that the electron transfer from the [4Fe-4S] clusters of DmsB to the Mo-bisMGD and the $E_{m,7} = -120$ mV [4Fe-4S] cluster of DmsB.

The structures of three proteins that have significant sequence similarity to DmsA have been solved. These are the $E. coli$ Me$_2$SO reductases from sequence similarity to DmsA have been solved. These are DmsB, (27, 28), and the catalytic subunits of the $E. coli$ Mo-bisMGD cofactor, the [4Fe-4S] cluster of DmsB, and engineered [3Fe-4S] clusters of appropriately mutagenized DmsA and DmsB. In the absence of detailed crystallographic data, these studies provide important information on the interactions between the prosthetic groups of DmsABC as well as on the electron transfer pathway from MQH$_2$ to Me$_2$SO.

**MATERIALS AND METHODS**

**Bacterial Strain and Plasmid**—The $E. coli$ strain and plasmids used in this study are listed in Table I. pDMS170 bears the wild-type dmsABC operon behind an fnr-dependent promoter and was generated by ligating the 4.8-kilobase pair EcoRI–SalI fragment from pDMS223 (4) into pBR322 that had previously been cut with XhoI to destroy the Mo(V)-[4Fe-4S] clusters of DmsA and engineered [3Fe-4S] clusters of appropriately mutagenized DmsA and DmsB. In the absence of detailed crystallographic data, these studies provide important information on the interactions between the prosthetic groups of DmsABC as well as on the electron transfer pathway from MQH$_2$ to Me$_2$SO.

**Growth of Cells and Preparation of Membrane Vesicles**—Cells were grown anaerobically in 20-liter batch cultures at 37 °C for 48 h on a glycerol-fumarate minimal medium (4, 31). Cells were harvested and washed in 100 mM MOPS and 5 mM EDTA (pH 7.0). Membranes were prepared by French pressure cell lysis and differential centrifugation as described previously (4, 6).

**Preparation of EPR Samples**—Membrane vesicles were suspended at a protein concentration of approximately 30 mg ml$^{-1}$ in 100 mM MOPS and 5 mM EDTA (pH 7.0). Dithionite-reduced (5 mM) samples were incubated under argon at 23 °C for 5 min. Oxidized samples were prepared by incubating membranes in the presence of 0.2 mM potassium ferricyanide for 2 min. Ferricyanide-oxidized, glycylglycine- and ferrocene-oxidized, glycylglycine- and methyl viologen and resorufin, indigotrisulfonate, indigodisulfonate, anthraquinone-2-sulfonic acid, phenosafranine, benzyl viologen, and methyl viologen. All samples were prepared in 3-mm internal diameter quartz EPR tubes and were rapidly frozen in liquid nitrogen-chilled ethanol before being stored under liquid nitrogen until use.

**EPR Spectroscopy**—Spectra were recorded using a Bruker ESP300 EPR spectrometer equipped with an Oxford Instruments ESR-900 flowing helium cryostat. Instrument conditions and temperatures were as described in the individual figure legends. Microwave power saturation data were fitted to the equation,

$$S = K \cdot \frac{P}{P_{0}} (1 + P/P_{0})^{n/2}$$

(Eq. 1)

where $S$ is the signal height, $K$ is a proportionality factor, $P$ is the microwave power, $P_{0}$ is the microwave power for half-saturation, and $n$ is the inhomogeneity parameter (35, 36).

### Table I

| Strain and plasmids | Description | Source |
|---------------------|-------------|--------|
| **Strain HB101**    | supE44 hsdS20 (r_h m_u) recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 met-l Strep^R | Laboratory collection |
| **Plasmids**        |             |        |
| pBR322              | Tet^R Amp^K | Amersham Pharmacia Biotech |
| pDMS170*            | pBR322 Amp^K (dmsABC)^+ | This study |
| pDMS160-C102S       | pBR322 Amp^K (dmsABC102S)^+ | Ref. 4 |
| pDMS160-C38S        | pBR322 Amp^K (dmsABC38S)^+ | Ref. 24 |
| pDMS160-176A        | pBR322 Amp^K (dmsABC176A)^+ | Ref. 19 |
| pDMS160-176A-C102S  | pBR322 Amp^K (dmsABC102S)^+ | This study |
| **pDMS170**         |             |        |

*pDMS170 was generated by ligating the 4.8-kilobase pair EcoRI–SalI fragment from pDMS223 (4) into pBR322 that had previously been cut with XhoI and NruI and self-ligated to destroy these sites.*
**RESULTS**

**Mo(V) EPR Spectra of the Wild-type, DmsA-C38S, and DmsA-R77S Enzymes**—Fig. 1 shows Mo(V) EPR spectra recorded at 75 K of membranes containing the wild-type (Fig. 1A), DmsA-C38S (Fig. 1B), and DmsA-R77S (Fig. 1C) mutant forms of DmsABC. All three spectra appear to be essentially identical, with g values of approximately 1.984, 1.980, and 1.960 ($g_1$, $g_2$, and $g_3$), suggesting that neither the DmsA-C38S nor the DmsA-R77S mutation has any significant effect on the overall structure of DmsABC.

**Effect of the DmsA-C38S and DmsA-R77S Mutations on the Redox Potentials for the Mo(V) and the Reduced [4Fe-4S] Cluster**—Fig. 1 also shows microwave power saturation curves recorded at various redox potentials for the $g = 1.98$ peak-trough versus $E_h$ values of approximately −24 mV. Given their lack of effect on the Mo(V) EPR line shape and their minor electrochemical effects, it is unlikely that the two DmsA mutations cause gross changes in the overall structure of DmsABC.

**Spin-Spin Interaction between the [4Fe-4S] Cluster and the Mo(V) of Wild-type DmsABC**—Camack and Weiner (2) originally identified an enhancement of the spin relaxation of the Mo(V) species of wild-type DmsABC that was consistent with it being due to an interaction between the [4Fe-4S] cluster and (ii) the Mo(V) of wild-type DmsABC. This enhancement was consistent with (i) the interacting [4Fe-4S] cluster being the $E_{m,7}$ of the [4Fe-4S] clusters of DmsB (2, 6). These data are consistent with (i) the interacting [4Fe-4S] cluster being the $E_{m,7}$ of the [4Fe-4S] clusters of DmsB (2, 6). These data are consistent with (i) the interacting [4Fe-4S] cluster being the $E_{m,7}$ of the [4Fe-4S] clusters of DmsB (2, 6). These data are consistent with (i) the interacting [4Fe-4S] cluster being the $E_{m,7}$ of the [4Fe-4S] clusters of DmsB (2, 6).
contributing independently to this interaction.

**Effect of the DmsA-C38S and DmsA-R77S Mutations on the Paramagnetic Interaction between the $E_{m,7}$ and $E_h$ Cluster of DmsB and the Mo(V) of DmsA**—Fig. 4 shows microwave power saturation curves for wild-type, DmsA-C38S, and DmsA-R77S DmsABC at potentials of approximately −20 and −150 mV. In the DmsA-C38S mutant, the microwave power saturation curve of the $E_h$ signal is essentially identical to that of wild-type DmsABC, saturating with a single $P_{1/2}$ of 1.2 mW. In contrast to the wild type, at $E_h = −155$ mV the Mo(V) signal saturates as an apparent single component with a $P_{1/2}$ of 2.0 mW and an inhomogeneity parameter (b) of 0.8. When microwave power saturation curves are recorded using redox poised samples (between $E_h = −20$ mV and $E_h = −180$ mV), b decreases to a lower limit of approximately 0.8 as the $E_{m,7} = −120$ mV [4Fe-4S] cluster becomes reduced (data not shown). Potentiometric studies indicate that the $E_{m,7}$ values of the DmsB [4Fe-4S] clusters (including the $E_{m,7} = −120$ mV cluster) remain unaltered in the DmsA-C38S mutant (23). Thus, the presence of the [3Fe-4S] cluster appears to significantly modulate the interaction between the $E_{m,7} = −120$ mV cluster of DmsB and the Mo(V) of the Mo-bisMGD cofactor of DmA.

In the DmsA-R77S mutant, the microwave power saturation curve of the $E_h$ is not shown at −18 mV sample is again very similar to that of the wild-type enzyme (Fig. 4). In this case, the $P_{1/2}$ is estimated to be 1.4 mW. However, at $E_h = −146$ mV, the saturation curve is essentially identical to that observed at −18 mV, indicating that the DmsA-R77S mutation eliminates the magnetic interaction between the Mo(V) and $E_{m,7} = −120$ mV cluster of DmsB. EPR spectra recorded at 12 K of redox-poised samples indicate that there is no detectable shift of the midpotential of the $E_{m,7} = −120$ mV cluster in the DmsA-R77S mutant (data not shown). When the potential dependence of the microwave power saturation curves is investigated, greater than 95% of the Mo(V) saturates with a low $P_{1/2}$, even at potentials as low as −200 mV (data not shown). Because of the disappearance of the Mo(V) signal at low $E_h$, it was not possible to investigate the effect of reduction of the two lowest potential [4Fe-4S] clusters (the $E_{m,7} = −240$ and −330 mV clusters) on the microwave power saturation properties or line shape of the
Mo(V) spectrum.

Interaction between Glycerol-inhibited Mo(V) and the Oxidized DmsA-C38S [3Fe-4S] Cluster—We have previously demonstrated that mutagenesis of the protein-molybdenum ligand of DmsA (Ser-176) generates a form of the Mo-bisMGD in which the molybdenum remains in the paramagnetic Mo(V) redox state at high \( E_a \) (19). We have also generated mutants of DmsB in which the [3Fe-4S] cluster coordinated primarily by Cys group III is replaced by a high potential [3Fe-4S] cluster (4, 6). By straightforward subcloning (see “Materials and Methods”), it was possible to generate a double mutant, DmsA\(^{S176A}S_{102SC}\), which at high \( E_a \) contains Mo(V) and the oxidized DmsB [3Fe-4S] cluster.

Fig. 6A shows EPR spectra recorded at 30 K and 0.2-mW microwave power of ferricyanide-oxidized membranes containing overexpressed DmsA\(^{S176A}B_{102SC}\) (Fig. 6A, i) and DmsA\(^{S176A}B_{102SC}\) (Fig. 6A, ii). In both cases, a Mo(V) signal was observed with \( g \) values of approximately 2.018, 1.982, and 1.961 (\( g_1 \), \( g_2 \), and \( g_3 \)), as previously reported (19). The minor features in the \( g \) = 2.00–2.03 region result from the incomplete broadening of the DmsB-C102S [3Fe-4S] cluster spectrum at the temperature and microwave power used to record the spectra of Fig. 6A. Fig. 6B shows microwave power saturation profiles of the Mo(V) spectra of DmsA\(^{S176A}B_{102SC}\) and DmsA\(^{S176A}B_{102SC}\). In both cases, the estimated \( P_{1/2} \) is 0.8 mW, indicating that the presence of the oxidized \( S = \frac{1}{2} \) [3Fe-4S] cluster of DmsB has no detectable effect on the microwave power saturation properties of the Mo(V) of DmsA.

**DISCUSSION**

We have demonstrated herein the effects of mutations of residues along the electron transfer pathway of DmsA on the EPR properties and redox chemistry of the Mo-bisMGD cofactor of DmsABC. Based on their effects on the Mo(V) EPR spectrum, it is clear that the DmsA-C38S and DmsA-R77S mutants have little effect on the environment or coordination sphere of the molybdenum and only minor effects on its redox chemistry. Given these results, it is unlikely that the mutations result in any gross modification of the protein structure that may result in significant differences in inter cofactor distances between the wild-type and mutant proteins. The DmsA-C38S and DmsA-R77S mutants both have significant effects on the observed Mo(V)-[4Fe-4S] interaction. These effects provide important insights into the electron transfer pathway from the [4Fe-4S] clusters of DmsB to the Mo-bisMGD cofactor of DmsA.

That the DmsA Cys-38 and Arg-77 residues do not contribute to the coordination sphere of the molybdenum of the Mo-
The DmsA-C38S and DmsA-R77S mutants appear to have minor, but opposite effects on the redox chemistry of the Mo-

bisMGD. The shift elicited by the DmsA-C38S mutant is a \( \Delta E_{m,7} = -20 \) mV shift of the Mo(IV/V) couple, whereas the shift elicited by the DmsA-R77S mutant is a \( \Delta E_{m,7} = -24 \) mV shift of the Mo(V/VI) couple. The [3Fe-4S] cluster of the DmsA-C38S mutant is reduced at \( E_{1/2} \) values where the Mo(V) is visible (it has an \( E_{m,7} \) of approximately 178 mV (23)) and therefore does not carry a formal charge. Thus, it is likely that subtle changes in structure caused by the introduction of a [3Fe-4S] cluster are responsible for the small change in the Mo(IV/V) \( E_{m,7} \). In the case of the DmsA-R77S mutant, the \( \Delta E_{m,7} = -24 \) mV shift in the Mo(V/VI) \( E_{m,7} \) may be rationalized in terms of the loss of the electron-withdrawing effect of the positive charge of the Arg residue. This suggests that the residues that contact the pterin ring systems may have an important role in defining the Mo(IV/V) and Mo(V/VI) \( E_{m,7} \) values. It should be noted that in the case of both mutations, the observed effects are on only one of the two molybdenum \( E_{m,7} \) values. Further mutagenesis studies will be necessary to confirm the role of pterin contact residues in defining the electrochemistry of the Mo-bisMGD.

Spin-spin interactions between EPR-visible prosthetic groups can provide important information on the topographical arrangement of these centers in multifactor enzymes. Of relevance to the data presented herein are the studies carried out on milk xanthine oxidase (36, 38–41). In this enzyme, there is a strong spin-spin interaction between various accessible forms of the Mo(V) of the molybdob-molybdopterin cofactor and one of the [Fe-S] clusters. This interaction results in both splitting of the EPR spectrum of the Mo(V) and in an enhancement of its spin relaxation rate at low temperatures that manifests itself as a significant increase in the \( P_{1/2} \). In the case of DmsABC, there is a strong spin-spin interaction between the Mo(V) species and the \( E_{m,7} = -120 \) mV [4Fe-4S] cluster that manifests itself as an increase in the Mo(V) \( P_{1/2} \) (Fig. 3), but does not result in a line shape change that can readily be interpreted as arising from a dipolar interaction.

A structure has recently become available of a bacterial protein similar in structure and sequence to xanthine oxidase. This protein, Desulfovibrio gigas aldehyde oxidoreductase (42), contains a molybdob-molybdopterin cytosine dinucleotide cofactor, and two [2Fe-2S] clusters. This enzyme has EPR properties similar to those of xanthine oxidase (43). It has been suggested that the cluster equivalent to the one interacting with the molybdenum in xanthine oxidase may be located approximately 15 Å from the molybdenum (44). In the structure of aldehyde oxidoreductase (42), one of the Cys ligands of this cluster is also hydrogen-bonded to the pterin. These observations bear interesting comparison with distance estimates for xanthine oxidase based on EPR analyses that are in the 8–25-Å range (41). However, it should be noted that the assignment of the interacting cluster in the structure of aldehyde oxidoreductase remains controversial (44). In another protein that is much more closely related to DmsA, E. coli FdhF, a [4Fe-4S] cluster is located approximately 13 Å from the molybdenum (26). No interaction between the [4Fe-4S] cluster and the molybdenum of FdhF has yet been reported.

Based on comparisons with xanthine oxidase and FdhF, a position for the \( E_{m,7} = -120 \) mV cluster of DmsA that is consistent with the observed spin-spin interaction between this cluster and the molybdenum would be equivalent to that of the interacting [2Fe-2S] cluster of xanthine oxidase or the [4Fe-4S] cluster of FdhF. However, it is clear from analyses of mutants of the DmsA Cys group that there is no EPR-detectable cluster coordinated by DmsA in the wild-type enzyme (23, 24). Also, mutants of DmsA Cys-38 and DmsA Arg-77 still contain a potentiometrically identifiable \( E_{m,7} = -120 \) mV cluster, further supporting the assertion that there is no cluster coordi-
nated by the DmsA Cys group. Based on qualitative comparisons with \textit{D. gigas} aldehyde oxidoreductase, it is therefore likely that the portion of DmsB containing the $E_{m,7} = -120 \text{ mV}$ cluster is located within approximately 15 Å of the molybdenum of DmsA.

One important distinction between the interaction reported herein and that observed in xanthine oxidase is the lack of apparent line shape change accompanying the enhancement of the spin relaxation of the Mo(V) signal. In xanthine oxidase, the line shape change manifests itself when the temperature of the sample is reduced to sufficiently slow down the spin relaxation rate of the interacting [2Fe-2S] cluster so that it is compatible with that of the Mo(V) species (39). The interacting [2Fe-2S] cluster of xanthine oxidase is a saturable (at 20 K) reduced [2Fe-2S] cluster (36) that displays readily interpretable behavior with increasing microwave power. In the case of DmsABC, the interacting species is the $E_{m,7} = -120 \text{ mV}$ [4Fe-4S] cluster of DmsB, which comprises half of a 2[4Fe-4S] ferredoxin motif with the $E_{m,7} = -50 \text{ mV}$ [4Fe-4S] cluster. At the potentials where the spin relaxation enhancement is observed, the $E_{m,7} = -120 \text{ mV}$ cluster is itself undergoing a complex interaction with the $E_{m,7} = -50 \text{ mV}$ cluster (2, 6). This interaction is equivalent to that observed in the bacterial 2[4Fe-4S] ferredoxins and results in an unsaturable EPR spectrum indicative of a very rapid spin relaxation rate (40, 45–47). Thus, in the interaction between the Mo(V) of DmsA and the $E_{m,7} = -120 \text{ mV}$ cluster of DmsB, the latter center is essentially unsaturable with increasing microwave power, and its relaxation rate at 30 K is very likely to be too high for the observation of significant splittings in the Mo(V) spectrum. A lack of quantifiable splitting of the DmsABC Mo(V) signal by the $E_{m,7} = -120 \text{ mV}$ [4Fe-4S] cluster of DmsB precludes a quantitative estimate of the intercenter distance based on the data presented herein.

We have demonstrated that incorporation of a [3Fe-4S] cluster coordinated by the DmsA Cys group modifies the interaction between the $E_{m,7} = -120 \text{ mV}$ cluster and the molybdenum (Fig. 4). This is consistent with (i) this cluster being located close to or on the vector joining the Mo(V) and the [4Fe-4S] cluster (for a dipolar interaction) and/or (ii) this cluster being on or close to the interaction pathway between the two centers (for an exchange interaction). The effect of the DmsA-R77S mutation (Fig. 4) clearly favors the second explanation, since presumably it has little effect on the distance between the two centers but is still able to eliminate the detectable interaction. Given that both the presence of a [3Fe-4S] cluster and the DmsA-R77S mutant both essentially eliminate electron transfer between DmsB and DmsA (23, 24), the data presented herein are consistent with the interaction pathway being equivalent to the electron transfer pathway. In the case of the interaction in xanthine oxidase, it has also been proposed that it occurs through a specific pathway through the protein and that it is also primarily exchange in nature (39).

Given the significant increase in the observed $P_{11}$ for the Mo(V) spectrum observed in the wild-type enzyme, we anticipated that it would be possible to detect a spin-spin interaction between the Mo(V) and either the reduced $S = 2$ or oxidized $S = \frac{1}{2}$ [3Fe-4S] cluster of the DmsA-C38S mutant. In the case of the glycerol-inhibited, ferricyanide-oxidized DmsA-C38S enzyme, there appears to be no enhancement of the Mo(V) spin relaxation and only a minor broadening of its EPR spectrum (Fig. 5). This result is somewhat surprising, since the [3Fe-4S] cluster of the DmsA-C38S mutant enzyme is, based on comparison with the structurally characterized FdhF (26), very likely to be located approximately 13 Å from the molybdenum.

Given the lack of significant interaction between the glycero-and the engineered [3Fe-4S] cluster in DmsA, it is not surprising that no interaction is observed between the Mo(V) of the DmsA-S176A mutant and the engineered [3Fe-4S] cluster of the DmsB-C102S mutant (Fig. 6). In this case, the lack of interaction may simply be due to the extra distance between the centers or perhaps due to the $E_{m,7} = -120 \text{ mV}$ cluster acting as a “shield,” preventing the observation of an interaction. In the DmsB-C102S mutant, the $E_{m,7} = -50 \text{ mV}$ [4Fe-4S] cluster of DmsB is converted to a [3Fe-4S] cluster (4, 6). As described above, in the wild-type enzyme, this cluster appears to form half of a 2[4Fe-4S] ferredoxin motif. Based on the structurally characterized bacterial 2[4Fe-4S] ferredoxins (48), this cluster is potentially a maximum of approximately 12 Å further away from the molybdenum of DmsA than the $E_{m,7} = -120 \text{ mV}$ cluster (this depends on the angle between the vector joining the $E_{m,7} = -120$ and $-50 \text{ mV}$ clusters and that joining the $E_{m,7} = -120 \text{ mV}$ cluster and the molybdenum center). Thus, an approximate maximum distance of 27 Å (12 plus 15 Å) is apparently too great for an interaction to be observed in DmsABC.

The data presented herein and previously reported data on the topology and electron transfer pathway of DmsABC (4–6, 9, 20, 23, 24, 49) allow a tentative model for electron transfer through the enzyme to be proposed. MQH$_2$ binding and oxidation occur at a single dissociable site in DmsC (20), which is conformationally linked to the $E_{m,7} = -50 \text{ mV}$ [4Fe-4S] cluster of DmsB (6). This cluster forms half of a 2[4Fe-4S] ferredoxin pair (2, 6) with the $E_{m,7} = -120 \text{ mV}$ [4Fe-4S] cluster. Electrons pass from MQH$_2$ through these two clusters and continue to the Mo-bisMGD cofactor via a vestigial [4Fe-4S] cluster binding domain defined by the N-terminal Cys motif of DmsA (24). This segment of the pathway is sensitive both to the presence of an engineered [3Fe-4S] cluster in the DmsA-C38S mutant and to mutation of a residue (DmsA Arg-77) that is in close juxtaposition to one of the pterins of the Mo-bisMGD (23, 24, 26). We have presented evidence herein that is consistent with this segment of the pathway being equivalent to the pathway of the magnetic interaction between the $E_{m,7} = -120 \text{ mV}$ [4Fe-4S] cluster and the molybdenum of the Mo-bisMGD cofactor. Given the potential position of DmsA Arg-77 in relation to one of the pterins, it is also very likely that one of the functions of this pterin is to provide a conduit for electron transfer to the molybdenum.

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