Multiple Pathways of Electron Transfer in Dimethyl Sulfoxide Reductase of Escherichia coli*

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The catalytic subunit of dimethyl sulfoxide (Me₂SO) reductase, DmsA, contains six blocks of sequence that are homologous to other members of the superfamily of prokaryotic molybdenzymes. The amino-terminal block contains 5 conserved residues (Cys²⁸, Cys⁴², Cys⁷⁵, Lys⁴⁸, and Arg⁷⁷). Site-directed mutagenesis of these residues did not alter membrane localization but in some cases less enzyme accumulated. The activity of Me₂SO reductase was monitored by measuring Me₅SO⁻ activity. These results indicate that the amino-terminal region is the most highly conserved. Three cysteine residues and 2 basic amino acids, Cys²⁸, Cys⁴², Lys²⁸, and Arg⁷⁷ of DmsA, are conserved in eight of the nine sequences. Only biotin sulfoxide reductase lacks this region. As biotin sulfoxide reductase is the only member of this group which does not accept electrons from an iron-sulfur subunit (Pierson and Campbell, 1990), it seems that these Cys and basic amino acids might participate in electron transfer from the iron-sulfur containing subunit to the catalytic subunit.

The activity of Me₂SO reductase can be monitored by: 1) measuring Me₂SO dependent anaerobic growth (Bilous and Weiner, 1985); 2) an artificial spectrophotometric assay using reduced benzyl viologen, BV⁻¹, as an electron donor (Weiner et al., 1988); 3) a physiological spectrophotometric assay using the quinol analogue 2,3-dimethyl-1,4-naphthoquinol, DMNH₂, as the electron donor (Sambasivarao and Weiner, 1991); and 4) the quinol-pool coupling electron paramagnetic resonance (EPR) assay (Rothery and Weiner, 1991). In this report, we have used site-directed mutagenesis to investigate the roles of residues Lys²⁸, Cys³⁸, Cys⁴², Cys⁷⁵, and Arg⁷⁷ in Me₂SO reductase and to examine the growth, expression, localization, and electron transfer properties of the mutant enzymes.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Plasmids—The E. coli strains and plasmids used in this study are listed in Table I.

Materials—Deoxyribonucleotides were synthesized on an Applied Biosystems 380 DNA Synthesizer in the DNA Core facility in the Dept. of Biochemistry, University of Alberta. DMN was a kind gift of Dr. A. Kröger, J. W. Goethe University, Frankfurt, Germany. Restriction endonucleases and modifying enzymes were from Life Technologies Inc. Sequenase and Sequenase reaction kits, Version 2.0, were from U. S. Biochemical Corp. The in vitro mutagenesis system was from Amer-
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ecDmsA:
ecDmsB:
ecDmsC:
ecDmsD:
ecDmsE:
ecDmsF:
ecDmsG:
ecDmsH:
ecDmsI:
ecDmsJ:
ecDmsK:
ecDmsL:
ecDmsM:
ecDmsN:
ecDmsO:
ecDmsP:
ecDmsQ:
ecDmsR:
ecDmsS:
ecDmsT:
ecDmsU:
ecDmsV:
ecDmsW:
ecDmsX:
ecDmsY:
ecDmsZ:

et al., 1990).

E. coli strains

Bacterial strains and plasmids

Strain or plasmid

Description

Source

E. coli strains

HB101

supE44

Boyer and Roulland-Dussoix (1969)

TG1

supE

Gibson (1984)

JM109

recA1, endA1, gyrA96, thi, hsdR17, supE44, relA1, lacIq, lacZAM15

Yanisch-Perron et al. (1985)

DSS301

TG1, 7104

Sambasivarao and Weiner (1991a)

Plasmids

pT7Z18R

Amp R

Pharmacia

pBR22

Tcr

Pharmacia

pDMS160

pBR322 Amp R (dmsABC) R

Rothery and Weiner (1991)

pDMS223

pBR322 Amp R (dmsABC) R

Rothery and Weiner (1991)

pK26A

pBR322 Amp R (dmsABC) R

This study

p26B

This study

pC38A

This study

pC38S

This study

pC42G

This study

pC42S

This study

pC75A

This study

pC75S

This study

pR77G

This study

pR77S

This study

Phage

M13K07

Pharmacia

sham. Protein assay standard and low molecular weight polyacrylamide gel electrophoresis standards were obtained from Bio-Rad. All other materials were reagent grade and were obtained from commercial sources.

Oligonucleotide-directed Mutagenesis—Oligonucleotide-directed mutagenesis of dmsA was performed as described by Rothery and Weiner (1991). After sequencing to confirm the mutation, the mutant DNA was subcloned into the wild-type dms operon on a 1-kilobase HindIII fragment to generate the mutant dmsA gene. The resultant plasmids were confirmed by restriction mapping and DNA sequencing.

Growth of Bacteria—For plasmid and phage manipulations and for preparation of inocula, cells were grown aerobically on Luria-Bertani Broth (Sambrook et al., 1989) or Terrific Broth (Tartof and Hobbs, 1987). E. coli cells were grown anaerobically at 37 °C on either glycerol/Me₂SO

FIG. 1. Sequence alignment of the amino-terminal region of E. coli DmsA (Bilous et al., 1988), E. coli NarZ (Blasco et al., 1990), E. coli NarG (Blasco et al., 1989), E. coli FdhA (Berg et al., 1991), FdhA from W. succinogenes (Bokranz et al., 1991), E. coli FdhF (Zinoni et al., 1986), FdhA from M. formicicum (Shuber et al., 1988), PsrA from W. succinogenes (Kraft et al., 1992), and E. coli Bisc (Pierson and Campbell, 1990). Conserved residues are shown in bold and the residues underlined were examined in this study.

Table 1

| Strain or plasmid | Description | Source |
|-------------------|-------------|--------|
| E. coli strains   |             |        |
| HB101             | supE44 hsdS20(rB mB) recA13 ara-14 procA2 lacY1 galK2 rpsL2O yfi-5 min-1 | Boyer and Roulland-Dussoix (1969) |
| TG1               | supE hsd55 thi Δ(lac-proAB) F' (traD38 proAB- lacI) lacZAM15 | Gibson (1984) |
| JM109             | recA1, endA1, gyrA96, thi, hsdR17, supE44, relA1, Δ(lac-proAB) F' (traD38 proAB- lacI) lacZAM15 | Yanisch-Perron et al. (1985) |
| DSS301            | TG1, dmsABC | Sambasivarao and Weiner (1991a) |
| Plasmids          |             |        |
| pT7Z18R           | Amp R lacZ | Pharmacia |
| pBR22             | Tcr Amp R | Pharmacia |
| pDMS160           | pBR322 Amp R (dmsABC) R | Rothery and Weiner (1991) |
| pDMS223           | pBR322 Amp R (dmsABC) R | Rothery and Weiner (1991) |
| pK26A             | pBR322 Amp R (dmsABC-Δ709BC-Δ793BC) | This study |
| p26B              | pBR322 Amp R (dmsABC-Δ709BC-Δ793BC) | This study |
| pC38A             | pBR322 Amp R (dmsABC-Δ709BC-Δ793BC) | This study |
| pC38S             | pBR322 Amp R (dmsABC-Δ709BC-Δ793BC) | This study |
| pC42G             | pBR322 Amp R (dmsABC-Δ709BC-Δ793BC) | This study |
| pC42S             | pBR322 Amp R (dmsABC-Δ709BC-Δ793BC) | This study |
| pC75A             | pBR322 Amp R (dmsABC-Δ709BC-Δ793BC) | This study |
| pC75S             | pBR322 Amp R (dmsABC-Δ709BC-Δ793BC) | This study |
| pR77G             | pBR322 Amp R (dmsABC-Δ709BC-Δ793BC) | This study |
| pR77S             | pBR322 Amp R (dmsABC-Δ709BC-Δ793BC) | This study |
| Phage             |             |        |
| M13K07            | Kan R | Pharmacia |
ampicillin (100 pg ml⁻¹), kanamycin (50 pg ml⁻¹), and streptomycin (100 µg ml⁻¹). Membranes were stored at -70 °C prior to use.

Preparation of Membrane Fractions—Membrane fractions were prepared by the method of Yamato et al. (1975) for use in expression studies and enzyme assays. This method gives a 2-fold enrichment of Me₂SO specific activity over washed membranes. For EPR analysis washed membrane fractions were prepared by French Pressure lysis and differential centrifugation as described by Cammack and Weiner (1990). Membranes were stored at -70 °C prior to use.

Enzyme Assays—Me₂SO reductase activity was determined by monitoring the Me₈SO- or TMAO-dependent oxidation of reduced benzyl viologen (BV⁻) as described by Cammack and Weiner (1990). The reduced DMN (DMNH₂) and Me₂SO- or TMAO-dependent oxidation of dithionite was assayed as described by Sambasivarao and Weiner (1991a). One unit of activity corresponds to 1 pmol of BV⁻ or dithionite oxidized min⁻¹.

Protein Determination and Polyacrylamide Gel Electrophoresis—Protein concentrations were estimated by a modification of the Lowry procedure (Markwell et al., 1978) using a Bio-Rad protein standard. Polyacrylamide gel electrophoresis of 12.5% acrylamide gels was carried out using the Bio-Rad Mini-gel system and a discontinuous SDS buffer system (Laemmli, 1970). Gels were stained with Coomassie Blue, destained, and the relative amount of DmsA protein was determined using a Joyce-Loebel Chromoscan 3 densitometer.

Electron Paramagnetic Spectroscopy—Samples were prepared as described by Cammack and Weiner (1990) from washed membranes of E. coli DSS301 cells grown on glycerol/fumarate medium. Sample protein concentrations were 30 mg ml⁻¹. Dithionite (5 mM) reduced samples were incubated under argon at 23 °C for 2 min. 25 mM Me₄SO or fumarate was added and the samples incubated for a further 2 min. Air-oxidized samples were vigorously stirred with a coiled stainless steel wire. Spectra were recorded using a Bruker ESP300 EPR spectrometer equipped with an Oxford Instruments ESR-900 flowing helium cryostat operating the Me₄SO reductase in E. coli cells harboring wild-type and mutant plasmids.

Preparation of Mutants—Five conserved residues were selected for analysis. Mixed mutagenic oligonucleotides (Table II) were used to replace Cys**₆₈**, Cys**₈²**, Cys**₂₅**, Lys**₂₈**, and Arg**₇₇** with serine or either alanine or glycine. Serine and alanine were chosen as replacements as they are unlikely to cause structural defects (Bordo and Argos, 1991). Glycine substitutions were obtained as a result of using mixed oligonucleotides.

Expression and Localization of Mutant Enzymes—In membrane preparations from E. coli HB101/pDMS158 (dmsABC) DmsA was recognized as a major 87-kDa polypeptide (Weiner et al., 1988). DmsA and DmsC could also be identified in the membranes but these subunits were less obvious due to co-migrating polypeptides and the diffuse staining of DmsC (Bilous and Weiner, 1988). To compare the expression/accumulation of DmsA from the mutant plasmids in HB101 we used densitometric scanning of Coomassie Blue-stained SDS-polyacrylamide gel electrophoresis gels (Table III). The amount of DmsA accumulated varied widely. In general, the serine substitutions accumulated DmsA to levels near the wild-type. With the exception of HB101/pK28A, the other alanine and glycine mutant plasmids accumulated lesser amounts of DmsA in the membrane. HB101/pC75A did not accumulate detectable DmsA. pC38S, pC42S, pC75S, and pR77S were selected for detailed study as they presented the most consistent pattern of expression.

In all but one case approximately 70% of the total Me₂SO reductase activity was localized to the cytoplasmic membrane with the remaining 30% found in the cytoplasm. This is similar to the distribution seen with wild-type enzyme activity (Sambasivarao et al., 1990). The exception was HB101/pR77G, which accumulated about 70% of the activity in the cytoplasmic fraction. Immunoblotting (not shown) confirmed a larger amount of DmsAB in the cytoplasmic fraction of HB101/pR77G than in HB101/pDMS160 suggesting that perturbation in the structure of DmsA had occurred altering membrane localization of the catalytic dimer.

We examined expression of the serine mutants in E. coli DSS301 which carries a total deletion of the dms operon (Sambasivarao and Weiner, 1991a). The expression levels of the pC38S, pC42S, and pR77S subunits correspond to only half the protein seen in HB101. DSS301/pK28A accumulated DmsA to levels comparable to the wild-type enzyme. The distribution of enzyme between membrane and cytoplasm was similar in DSS301 and HB101.

**RESULTS**

**Preparation of Mutants**—Five conserved residues were selected for analysis. Mixed mutagenic oligonucleotides (Table II) were used to replace Cys**₆₈**, Cys**₈²**, Cys**₂₅**, Lys**₂₈**, and Arg**₇₇** with serine or either alanine or glycine. Serine and alanine were chosen as replacements as they are unlikely to cause structural defects (Bordo and Argos, 1991). Glycine substitutions were obtained as a result of using mixed oligonucleotides.

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| Mutant  | Codon change | Mutagenic oligonucleotide |
|---------|--------------|----------------------------|
| K28S    | AAG → CAG    | 3'−CAGACGACGCGGTATAC−5' |
| R28S    | AAG → TGG    | 3'−CAGGTAACGCGGTATAC−5' |
| C38S    | TCT → TTC    | 3'−CAGGTAACGCGGTATAC−5' |
| C42S    | TCC → TGC    | 3'−CAGGTAACGCGGTATAC−5' |
| C75A    | TGC → AGC    | 3'−CAGGTAACGCGGTATAC−5' |
| R77G    | CTT → GGT    | 3'−CAGGTAACGCGGTATAC−5' |

**TABLE III**

| Plasmid | Expression of DmsA in |
|---------|-----------------------|
|         | E. coli                  | E. coli                  |
|         | HB101                  | DSS301                  |
| pBR322  | 3.3                     | 0                       |
| pDMS160 | 12.9                    | 13.9                    |
| pK28A   | 12.1                    | ND                      |
| pC38A   | 11                      | 15.3                    |
| pC38S   | 9.4                     | ND                      |
| pC42G   | 10.7                    | 4.8                     |
| pC42S   | 8.4                     | 4.8                     |
| pC75A   | 0                       | ND                      |
| pC75S   | 8.3                     | 9                       |
| pR77G   | 5.9                     | ND                      |
| pR77S   | 16                      | 5.9                     |

* Percentage of DmsA relative to total protein was determined by densitometry scans of polyacrylamide gels. 45 µg of membrane protein was loaded per lane.

ND, not determined.
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measure of the function of the mutant enzymes. DSS301 carrying the mutant plasmids were grown anaerobically on glycerol/Me₂SO medium producing the doubling times shown in Table IV. Only pDMS160, pK28A, pK28S, and pC75S were able to support growth of DSS301, although cells carrying pC75S grew much slower.

We examined growth of HB101 harboring these dms plasmids (Table IV). Although HB101 has a chromosomal copy of Me₂SO reductase, the mutants that did not support growth in DSS301 inhibited the growth of HB101. The overexpressed mutant subunits compete with the chromosomally encoded wild-type DmsA for assembly into the holoenzyme resulting in this inhibition. Such effects have previously been reported in studies of Me₂SO reductase (Rothery and Weiner, 1991) and fumarate reductase (Weiner et al., 1986; Westenberg et al., 1990).

Electron Transfer from the Artificial Electron Donor Benzyl Viologen—Membrane fractions from HB101 harboring the dms plasmids were assayed for Me₂SO reductase activity using BV⁺ as electron donor and Me₂SO or TMAO as oxidant (Table V). All of the mutant enzymes displayed a specific activity higher than HB101/pBR322. HB101/pC42S had the lowest specific activity (35 units mg⁻¹) which was only slightly enhanced over the specific activity of HB101/pBR322 (29 units mg⁻¹). The HB101/pC42S activity may represent a combination of chromosomal dms expression and excess pC42S expression but the results suggest that this residue plays a role in BV⁺ oxidase activity. When the activities were normalized to the amount of DmsA in the membrane (Table V), HB101/pC42S had the lowest activity again suggesting that this mutation had decreased the efficiency of Me₂SO reductase activity.

All of the plasmids expressed BV⁺-dependent Me₂SO reductase activity in DSS301 (Sambasivarao and Weiner, 1991a). The specific activity of DSS301/pDMS160 was 79 units mg⁻¹ (TMAO as oxidant), whereas DSS301/pC42S displayed the lowest specific activity (14 units mg⁻¹). This activity must come from the mutant plasmid as DSS301 has a specific activity of 0.2 units mg⁻¹ with TMAO. As in HB101, the other mutant plasmids demonstrated intermediate activities.

The ratio of TMAO to Me₂SO activity was generally between 7 and 9, in agreement with the reported activity ratio (Sambasivarao and Weiner, 1991a). Two of the mutants had substrate activity ratios outside this range (HB101/pK28S had a ratio of 10.5 and HB101/pC42S had a ratio of 5) but it is unlikely that these variations represent a difference in substrate utilization. Together these results suggest that of these 5 conserved residues only Cys⁴⁶ may play a role in BV⁺ oxidase activity.

Electron Transfer from the Quinol Analogue Dimethylnaphthoquinol—DMNH₂ is a quinol analog previously used to analyze electron transfer within fumarate reductase (Weiner et al., 1986) and Me₂SO reductase (Sambasivarao and Weiner, 1991a). It is reactive only with the holoenzyme forms of these reductases, in contrast to benzyl viologen which will transfer electrons to either the catalytic dimer or holoenzyme. DMNH₂-dependent oxidation of TMAO and Me₂SO by HB101 membranes is shown in Table V. All mutants, except Arg⁷⁷-Ser, display specific activities greater or equal to HB101/pBR322. HB101/pC38S and HB101/pC42S have low levels of activity and it is unclear if this activity results from chromosomal or plasmid expression. HB101/pR77S has very low activity suggesting that this residue is essential for DMNH₂ oxidase activity.

All mutant enzymes possessed DMNH₂ activity in DSS301 where the endogenous DMNH₂:TMAO activity is only 0.025 units mg⁻¹. DSS301/pC38S and DSS301/pC42S had specific activities of 0.3 and 0.2 units mg⁻¹, respectively, suggesting that these residues are not essential for DMNH₂ oxidase activity but play a role in the efficiency of the reaction. Similarly, DSS301/pR77S had a very low specific activity of 0.05 units mg⁻¹ suggesting that this residue, although not essential, has a major effect on DMNH₂ oxidase activity. Interestingly, the ratio of TMAO to Me₂SO activity with DMNH₂ as electron donor was near 2 compared to 5–10 for BV⁺ suggesting that the mechanisms of the benzyl viologen and DMN reactions differ.

### Table IV

| Plasmid     | E. coli DSS301 | E. coli HB101 |
|-------------|----------------|--------------|
| pBR322      | NG⁺           | 6.7⁺         |
| pDMS160     | 2.0           | 5.0          |
| pK28A       | 2.4           | 5.2          |
| pK28S       | 2.8           | 5.6          |
| pC38A       | NG            | NG           |
| pC38S       | NG            | 10.0         |
| pC42G       | NG            | NG           |
| pC42S       | NG            | 10.0         |
| pC75A       | NG            | 5.5          |
| pC75S       | 7.1           | 7.7          |
| pR77G       | NG            | NG           |
| pR77S       | NG            | 8.6          |

⁻ NG, no growth on glycerol/Me₂SO minimal medium.
⁶ Doubling times were determined from measurements of cell densities in glycerol/Me₂SO minimal medium using a Klett-Summerson spectrophotometer equipped with a number 66 filter.

### Table V

| Plasmid     | Benzyloviologen | TMAO Normalised BV⁺:TMAO⁺ | TMAO DMN | DMN TMAO Q-pool coupling⁺ |
|-------------|-----------------|--------------------------|----------|--------------------------|
| pBR322      | 29 ± 3          | 0.5 ± 0.07               | 0.8 ± 0.07 | 0.5 ± 0.05 | 1.9 | + |
| pDMS160     | 190 ± 18        | 11 ± 1                   | 1400 ± 100 | 5.5 ± 1.5 | 2.1 ± 0.3 | 2.6 | + |
| pK28A       | 179 ± 66        | 17 ± 5                   | 1000 ± 100 | 8.9 ± 1.3 | 2.0 ± 0.5 | 4.5 | + |
| pC38S       | 71 ± 9          | 9 ± 1                    | 600 ± 20  | 1.6 ± 0.1 | 0.5 ± 0.2 | 2.2 | - |
| pC42S       | 35 ± 9          | 7 ± 2                    | 500 ± 50  | 1.3 ± 0.5 | 0.7 ± 0.4 | 1.9 | + |
| pC75S       | 84 ± 14         | 12 ± 3                   | 1000 ± 200 | 2.2 ± 0.6 | 1.1 ± 0.1 | 2.0 | + |
| pR77S       | 138 ± 34        | 16 ± 3                   | 700 ± 200 | 0.3 ± 0.1 | 0.1 ± 0.04 | 2.9 | - |

⁻ Membranes were prepared from HB101 cells grown on glycerol/fumarate medium. BV⁺ substrate enzyme activities are expressed in units of pmol of BV⁺ oxidized min⁻¹ mg⁻¹ protein. DMN, substrate enzyme activities are expressed in units of micromole of dithionite oxidized min⁻¹ mg⁻¹ protein.
⁶ BV⁺:TMAO activities were normalized for the amount of DmsA expression determined by densitometry. One unit corresponds to 1 pmol of BV⁺ oxidized min⁻¹ mg⁻¹ DmsA⁻¹.
⁺ Membranes were prepared from DSS301 cells grown on glycerol/fumarate medium and the ability of Me₂SO reductase to oxidize the menaquinol pool was examined.
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**Electron Dansfer Pathways**

**Fig. 2.** A, EPR spectra of DSS301/pBR322 membranes. The spectra are of membranes from glycerol/fumarate-grown DSS301/pBR322: (i) reduced with 5 mM dithionite; (ii) reduced with 5 mM dithionite and treated with 25 mM Me₂SO; (iii) reduced with 5 mM dithionite and treated with 25 mM fumarate; (iv) air oxidized. B, EPR spectra of DSS301/pDMS160. The spectra are of membranes from glycerol/fumarate-grown DSS301/pDMS160: (i) reduced with 5 mM dithionite; (ii) reduced with 5 mM dithionite and treated with 25 mM Me₂SO; (iii) reduced with 5 mM dithionite and treated with 25 mM fumarate; (iv) air oxidized.

**Ability of Wild-type and Mutant Enzymes to Oxidize the Menaquinol Pool**—The membrane menaquinol pool is in equilibrium with several terminal reductases including fumarate and Me₂SO reductases. Functional Me₂SO reductase is able to draw electrons from fumarate reductase through the menaquinol pool to reduce Me₂SO and the reverse also is true. We can follow this reaction using EPR spectroscopy of the endogenous [Fe-S] clusters in these proteins (Rothery and Weiner, 1991). DSS301 membranes contain fumarate reductase which can be used to determine if plasmid encoded Me₂SO reductase is able to catalyze Me₂SO-dependent menaquinol oxidation. We have used this quinol-pool coupling (Q-pool coupling) assay to determine whether mutants of Me₂SO reductase are able to accept electrons from the endogenous quinol pool (Table V). Fig. 2A shows the spectra obtained from E. coli DSS301/pBR322 membranes. When reduced with dithionite the prominent features are a peak at g = 2.03 and a peak trough at g = 1.94, which are characteristic of the reduced FR1 [2Fe-2S] center of fumarate reductase (Johnson et al., 1988). Addition of 25 mM Me₂SO or TMAO (data not shown) does not change the spectrum of these membranes but the addition of 25 mM fumarate causes the FR1 features to diminish and a sharp peak at g = 2.02 with a broad trough immediately upfield to appear. These new features are characteristic of the oxidized [3Fe-4S] center of fumarate reductase, FR3 (Johnson et al., 1988). In Fig. 2B the spectra of membranes from DSS301/pDMS160 are shown. In the dithionite-reduced sample the fumarate reductase signal is still present but a new peak at g = 1.99 and a trough at g = 1.88 are also visible. These features are part of the spectrum of reduced Me₂SO reductase (Cammack and Weiner, 1990). When 25 mM Me₂SO (or TMAO) is added the dithionite-reduced features of both Me₂SO reductase and fumarate reductase are diminished while the oxidized FR3 signal appears. Similarly, the addition of fumarate to reduced membranes also causes both enzymes to become oxidized. This coupling demonstrates...
that Me$_2$SO reductase is able to interact with and accept electrons through the quinol pool and pass them on to the substrate.

Fig. 3A shows EPR spectra of the DSS301/pK28S mutant membranes which have been reduced with dithionite. The dithionite-reduced spectrum is a composite of fumarate reductase (DmsABC, Bilous (1986); and HycCD, Sauter (1990)), nitrate reductases (NarGHJI, Blasco et al. (1992)), and the formate dehydrogenases from E. coli (FdhABCD, Bokranz et al. (1989); NarZYWV, Blasco et al. (1991); Wollinella succinogenes polysulfide reductase (FsdABC, Krafft et al. (1992)), and the formate dehydrogenases from E. coli (FdhAB, FdhHI, Berg et al. (1991); FDNH-FdhF, Zinoni et al. (1986); and HycCD, Sauter et al. (1992)). Methanobacterium formicicum (FdhAB, Schubert et al. (1986) and FdhC, White and Ferry (1992)) and W. succinogenes (FdhABCD, Hokrnan et al. (1991)). Members typically contain three subunits: a large molybdopterin containing catalytic subunit (e.g. DmsA, NarG), a cysteine-rich electron transfer subunit ligating [Fe-S] clusters (e.g. DmsB, NarH), and a membrane anchor subunit (e.g. DmsC, NarI). Multiple amino acid sequence alignment clearly shows the relationship of the catalytic and electron transfer subunits while the anchor subunit appears to be far less conserved. The catalytic subunits contain several blocks of sequence homology which are present in all members of the family and presumably some of these regions participate in complexing the molybdopterin cofactor. The order of these blocks from amino to carboxyl terminus is constant but their relative spacing varies greatly (Weiner et al., 1992). Interestingly, biotin sulfoxide reductase (BisC, Pierson and Campbell (1990)), which receives its reducing equivalents from a thioredoxin-like soluble protein, lacks the amino-terminal block (Fig. 1). The other eight enzymes require the Cys-rich electron transfer subunit and this suggested to us that the first block may be necessary for electron transfer to the catalytic subunit. The results presented herein confirm this hypothesis.

The eight homologous proteins contain 3 Cys residues equivalent to Cys$^{38}$, Cys$^{62}$, and Cys$^{75}$ of DmsA (Fig. 1). DmsA contains an additional conserved Cys residue at position 34 but this amino acid is replaced by a His in nitrate reductase. Changing Cys$^{34}$ of DmsA to His does not appear to affect the properties of Me$_2$SO reductase. The proteins also contain conserved basic residues corresponding to Lys$^{28}$, Arg$^{77}$, and Arg$^{150}$ of DmsA. Mutagenesis of Arg$^{90}$ to Ser$^{1}$ did not alter any measurable activity of Me$_2$SO reductase. Additional conserved residues including Gly$^{78}$ and Pro$^{104}$ were not mutated due to their potential structural role. The roles of Lys$^{28}$, Cys$^{38}$, Cys$^{62}$, Cys$^{75}$, and Arg$^{77}$ in Me$_2$SO reductase were examined by site-directed mutagenesis.

**Lys$^{28}$**—Mutation to the neutral amino acids Ser or Ala did not alter the growth, expression, or catalytic activities of Me$_2$SO reductase indicating that this conserved residue is not important for the activities we measured.

**Cys$^{38}$**—Mutation to Ser produced an enzyme which could not support growth on Me$_2$SO although the membrane localization was wild-type and accumulation of mutant enzyme was greatly impeded. The enzyme catalyzed BV$^{-}$ oxidase activity at $-50\%$ of wild-type and had low DMNHz oxidase activity. It could not accept electrons from the menaquinol pool.

**Cys$^{62}$**—Cys$^{62}$-Ser could not support growth on Me$_2$SO. Again, the membrane localization was wild-type and accumulation of this enzyme was not greatly reduced. This enzyme had the poorest BV$^{-}$ oxidase activity and low DMNHz activity suggesting that Cys$^{62}$ was necessary for optimal activity. Cys$^{62}$-Ser could not accept electrons from the menaquinol pool.

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2 C. A. Trieber and J. H. Weiner, unpublished results.

3 R. A. Rothery and J. H. Weiner, unpublished results.
Cys75—Mutation to Ser produced an enzyme which supported growth on MegSO. The membrane localization was normal. It displayed relatively high benzyl viologen and DMNHz activity and could transfer electrons from the menaquinol pool.

Arg77—This residue was essential for growth and both DMNHz and menaquinol oxidative activities. The Arg77-Ser mutant had near wild-type benzyl viologen activity, localization, and accumulation.

We have combined these results into a working model which is shown in Fig. 4. In this model the BV oxidase activity requires both the DmsA and B subunits, but not DmsC. It is not clear if any of the four [4Fe-4S] centers are needed for this reaction but our preliminary data suggests that at least the clusters ligated primarily by Cys groups I and II in DmsB are not functional and unable to transfer electrons back to the menaquinol pool, but in Cys1', Cys4', and Arg80. In all cases the enzyme assembled functional [4Fe-4S] clusters which could be reduced by dithionite and which could pass their electrons back to the menaquinol pool, but in Cys38, Cys42, and Arg77 the enzyme was unable to pass these electrons on through DmsA.

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