JOURNAL OF BACTERIOLOGY, Apr. 1988, p. 1505–1510
0021-9193/88/041505-06$02.00/0

Vol. 170, No. 4

Purification and Properties of *Escherichia coli* Dimethyl Sulfoxide Reductase, an Iron-Sulfur Molybdoenzyme with Broad Substrate Specificity

JOEL H. WEINER,* DOUGLAS P. MACISAAC, RUSSELL E. BISHOP, AND PETER T. BILOUS

Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7

Received 10 September 1987/Accepted 30 December 1987

Dimethyl sulfoxide reductase, a terminal electron transfer enzyme, was purified from anaerobically grown *Escherichia coli* harboring a plasmid which codes for dimethyl sulfoxide reductase. The enzyme was purified to >90% homogeneity from cell envelopes by a three-step purification procedure involving extraction with the detergent Triton X-100, chromatofocusing, and DEAE ion-exchange chromatography. The purified enzyme was composed of three subunits with molecular weights of 82,600, 23,600, and 22,700 as identified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The native molecular weight was determined by gel electrophoresis to be 155,000. The purified enzyme contained 7.5 atoms of iron and 0.34 atom of molybdenum per mol of enzyme. The presence of molybdopterin cofactor in dimethyl sulfoxide reductase was identified by reconstitution of cofactor-deficient NADPH nitrate reductase activity from *Neurospora crassa nit-1* mutant and by UV absorption and fluorescence emission spectra. The enzyme displayed a very broad substrate specificity, reducing various *N*-oxide and sulfoxide compounds as well as chlorate and hydroxylamine.

The facultative anaerobe *Escherichia coli* is capable of anaerobic growth by glycolysis or by oxidative phosphorylation with alternate terminal electron acceptors. Generation of a proton motive force coupled to respiration has been well established for nitrate (16) and fumarate (9). In addition, trimethylamine *N*-oxide (TMAO) is known to serve as a terminal electron acceptor for anaerobic growth (3), and we have shown that dimethyl sulfoxide (DMSO) is also utilized in this way (4, 5). Nicotinamide *N*-oxide (C. Diaz and R. Meganathan, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, K122, p. 150) and tetrahydrothiophene oxide (25) have recently been shown to serve as terminal electron acceptors.

Several TMAO reductases have been detected in *E. coli* and are distinguished by their subcellular location, inducibility, and genetic parameters (28, 31, 34); however, whether these represent distinct enzymes is unclear. With the exception of the reductases associated with growth on fumarate and nitrate, it is also uncertain whether separate enzymes are induced for each terminal electron acceptor or a common enzyme can utilize a number of related substrates.

We have recently identified and cloned the genes for a DMSO reductase which is genetically distinct from known terminal reductases (6). In this paper we use membranes, isolated from cells harboring the cloned operon, for the purification of the enzyme to homogeneity. The purified enzyme has a very broad substrate specificity and may account for the ability of *E. coli* to grow anaerobically on a diverse array of *S*- and *N*-oxide compounds.

MATERIALS AND METHODS

Chemicals. 2-Picoline *N*-oxide, 3-picoline *N*-oxide, 4-picoline *N*-oxide, 3-hydroxypyridine *N*-oxide, pyridine *N*-oxide, tetramethylene sulfoxide, methyl phenyl sulfoxide, and phenyl sulfoxide were obtained from Aldrich Chemical Co., Milwaukee, Wis. Octaethyleneglycol dodecyl ether ($C_{12}E_8$) was obtained from Nikko Chemical Co., Tokyo, Japan. All other chemicals and detergents were purchased from Sigma Chemical Co., St. Louis, Mo.

Bacterial strains and plasmids. *E. coli* HB101 (F^- *hsdR hsdM pro leu gal lac thi recA rpsL*) was transformed with plasmid pDMS159, an Ap^r recombinant pBR322 plasmid carrying the *E. coli* chromosomal DNA fragment coding for the entire DMSO reductase operon (6). *Neurospora crassa nit-1* mutant was obtained from the Fungal Genetic Stock Center, University of Kansas Medical Center, Kansas City, and used for the identification of molybdenum cofactor.

Growth of cells and preparation of the crude envelope fraction. *E. coli* HB101(pDMS159) was grown anaerobically in glycerol-fumarate minimal medium (32) containing 100 μg of ampicillin per ml for 46.5 h at 37°C in 19-liter carboys or a 300-liter fermentor (P. E. C. Fermenter, Chemap Ltd., Mannedorf-Zurich, Switzerland). Cells were harvested with a Pellicon apparatus equipped with a polysulfone PTHK filter (Millipore Corp., Bedford, Mass.) or a Sharples high-speed centrifuge, frozen in liquid N_2 and then stored at −70°C until required. Typical yields were 1 g (wet weight) of cells per liter of medium. In a typical preparation, 100 g of cell paste was washed with 30 mM Tris hydrochloride, pH 8.0 (10 ml/g), centrifuged at 10,000 × *g* for 10 min, and suspended in a minimal volume of 0.2 M sodium phosphate, pH 6.8, containing phenylmethylsulfonyl fluoride (1 mM). The cells were lysed in a French pressure cell, and crude membranes were prepared as previously described (4).

Enzyme solubilization. For analytical studies, crude membranes (45.5 mg of protein per ml in 0.2 M sodium phosphate, pH 6.8) were incubated with detergents at a detergent/protein ratio of 0.8 (wt/wt). NaCl, when used, was added to a final concentration of 1 M. Solubilization mixtures were incubated for 30 min at 4°C with occasional mixing and then centrifuged at 371,000 × *g* for 15 min at 4°C in a TL100 tabletop ultracentrifuge (Beckman Instruments, Inc., Fullerton, Calif.). The supernatant was removed and used for enzyme assays.

Purification of DMSO reductase. For preparative extraction of DMSO reductase, Triton X-100 was added to the

* Corresponding author.

crude envelope preparation (800 mg of protein, 45.5 mg/ml) at a detergent/protein ratio of 0.8 (wt/wt). The mixture was incubated for 30 min at 4°C with occasional agitation. The extract was centrifuged at 150,000 × *g* for 60 min at 4°C and applied to a 200-ml column (3.2 by 26.0 cm) of Sephadex G-25 equilibrated with 0.025 M imidazole hydrochloride, pH 7.4, containing 0.1% Triton X-100. The colored void volume fractions from the G-25 column were pooled and subsequently chromatofocused on a 125-ml column (2.1 by 36.0 cm) of Pharmacia PBE^{TM}94 (prepared per the manufacturer's instructions) equilibrated with 0.025 M imidazole hydrochloride, pH 7.4, containing 0.1% Triton X-100. Sample material was eluted with 14 bed volumes of Polybuffer 74-hydrochloride diluted 1:8, adjusted to a pH of 4.0 and containing 0.1% Triton X-100. Peak fractions of DMSO reductase activity (80 ml) were concentrated approximately 20-fold in an ultrafiltration cell equipped with a PM30 filter (Amicon Corp., Lexington, Mass.). The concentrated sample was applied to a 60-ml column (1.5 by 34.0 cm) of Sephadex G-25 equilibrated with 20 mM histidine hydrochloride, pH 6.8, containing 10% glycerol, 0.5 mM EDTA, and 0.5 mM dithiothreitol (buffer H) and eluted with the same buffer. Colored void volume fractions were applied to a 75-ml (2.5 by 15.5 cm) column of DEAE-cellulose DE52 (Whatman, Inc., Clifton, N.J.), prepared as described by the manufacturer and equilibrated in buffer H. The column was washed with 3 bed volumes of buffer H and then 3 bed volumes of buffer H containing 400 mM KCl. DMSO reductase activity was subsequently eluted with buffer H containing 100 mM KCl and 0.1% Triton X-100.

Assay of reductase activity. Assays of reductase activity were carried out as previously described (4) by measuring the substrate-dependent oxidation of reduced benzyl viologen in open cuvettes. DMSO and TMAO were added to a final concentration of 10 mM in the assay. One unit corresponds to 1 μmol of benzyl viologen oxidized per min. The K_m values were determined from Lineweaver-Burke double-reciprocal plots. In experiments to measure substrate specificity, the substrates were used at the concentrations indicated in Table 2.

Estimation of native molecular weight. The method of Ferguson (12) was used to determine native molecular weight of proteins, using gels of four different acrylamide concentrations (4.5, 6, 7.5, and 9%).

Native enzyme molecular weight was also estimated by gel chromatography, using a Sephacryl S300 superfine (Pharmacia, Uppsala, Sweden) column (1.5 by 25.5 cm) equilibrated with buffer H containing 0.1% Triton X-100.

SDS-polyacrylamide gel electrophoresis. Samples were analyzed on sodium dodecyl sulfate (SDS)-polyacrylamide slab gels of 22 by 15 by 0.15 cm with an acrylamide gradient of 12 to 17%. The discontinuous SDS buffer system of Laemmli was used (22). Gels were electrophoresed for 180 mA-h and stained with Coomassie brilliant blue R-250.

Spectral determinations. Visible and UV spectra were obtained with a Varian DMS100S spectrophotometer equipped with a DS15 data station or in a Cary 219 (Varian, Palo Alto, Calif.) spectrophotometer. Protein samples were diluted to 0.8 mg/ml in 0.2 M sodium phosphate, pH 6.8. Fluorescence spectra were determined on a Perkin-Elmer MPF-44B spectrofluorimeter. For spectral studies, DMSO reductase was purified with $C_{12}E_8$ as detergent because of its low absorption in the UV region (13).

Mo cofactor isolation and identification. Molybdenum cofactor associated with DMSO reductase was identified by the complementation of cofactor-deficient NADPH-nitrate reductase produced by the *nit-1* mutant of *N. crassa* (15). Mycelia were prepared from *N. crassa nit-1* as described by Amy and Rajagopalan (1) and harvested as described by Seki et al. (30). Crude extracts were prepared by lysing the mycelia with a Teflon homogenizer and centrifuging the lysate at 27,000 × *g* for 10 min. The supernatant was stored at −70°C until required. Crude molybdenum cofactor was isolated from either xanthine oxidase (Sigma) or DMSO reductase by denaturing the enzymes (2.8 mg of protein per ml) at 90 ± 5°C for 1 min followed by rapid cooling to 0°C. Denatured protein was removed by centrifugation at 15,600 × *g* for 2 min, and the supernatant was used for the reconstitution of nitrate reductase activity. The reconstitution procedure outlined by Seki et al. (30) was used with 10 mM sodium molybdate added to the reconstitution mixture. Reconstituted NADPH-nitrate reductase activity was determined by the method of Garrett and Nason (14), using NADPH as electron donor and nitrate as acceptor, and the quantity of nitrite produced was determined by the diazo-coupling assay of Nason and Evans (26).

For spectral sudies, the cofactor was released from DMSO reductase by boiling 0.1 ml of enzyme solution (2 mg of protein per ml, adjusted to pH 2.5 with 1 N HCl) for 20 min in the presence of 0.1% KI and 0.05% I_2 (18). Insoluble material was removed by centrifugation at 35,000 × *g* for 10 min in a Beckman Airfuge, and the entire supernatant was used for spectral studies. Spectra were determined in 50 mM sodium phosphate buffer, pH 6.8.

Analytical methods. Protein was determined by a modified Lowry procedure (23) or by the Bradford method (7), using bovine serum albumin (Bio-Rad Laboratories, Richmond, Calif.) as standard. Metals were determined with an inductively coupled plasma atomic emission spectrometer (ARL model 35000).

RESULTS

Solubilization of DMSO reductase from membranes. DMSO reductase from *E. coli* is a membrane-bound enzyme induced by anaerobic growth on fumarate (4–6). The enzyme was not released from the membrane by washing with low (distilled water)- or high (0.5 M sodium phosphate)-ionic-strength buffer or with EDTA (10 mM). A number of ionic and nonionic detergents were tested for their effectiveness in solubilizing the enzyme. The ionic detergent sodium deoxycholate (in the absence of salt) and the nonionic detergents Triton X-100 and Nonidet P-40 were effective in releasing the enzyme from the membrane (>87% of membrane-bound activity solubilized). Other nonionic detergents tested, Brij35, Brij38, and Tween 80, were found to be poor at solubilizing the enzyme from the membrane. The solubilization of enzyme activity by the various detergents tested closely paralleled the solubilization pattern determined for several membrane-bound enzymes (11, 35), suggesting that DMSO reductase was also an intrinsic membrane enzyme. Although $C_{12}E_8$ was not as effective as the other nonionic detergents (approximately 70% of the membrane-bound activity can be solubilized), it was used for spectral studies because of its low UV absorption properties compared with Trition X-100.

Purification of DMSO reductase. Initial attempts to purify the enzyme from membranes of *E. coli* HB101 were unsuccessful due to the low levels of activity in the cell and instability of the enzyme during isolation. We recently constructed a recombinant pBR322 plasmid (pDMS159) carrying the genes for DMSO reductase (6). Cells harboring

pDMS159 expressed eight- to ninefold-higher levels of enzyme activity than wild-type cells, which assisted purification.

The protocol developed to purify DMSO reductase is summarized in Table 1. Enzyme activity was extracted from crude envelope preparations with the nonionic detergent Triton X-100. The detergent extraction appeared to cause activation and resulted in a higher total activity than determined for the crude envelope fraction. Chromatofocusing in the presence of Triton X-100 was found to be a very useful step for purifying the enzyme. Activity eluted as a symmetrical peak at pH 4.35, and in some preparations peak fractions were judged to be nearly homogeneous based on SDS-polyacrylamide gel electrophoresis. The final step made use of an unusual property of the enzyme. Pooled fractions from the chromatofocusing column were applied to a Sephadex G25 column equilibrated with buffer devoid of detergent. The activity peak, which was not visibly aggregated, was immediately applied to a DEAE-cellulose column equilibrated with buffer lacking detergent. The enzyme adsorbed to the top of the column and the column could be washed with buffer containing up to 1 M KCl, which removed contaminants without eluting enzyme activity. Nearly homogeneous enzyme could then be eluted by adding 0.1% Triton X-100 to the elution buffer. Enzyme was recovered with a 55% yield and was purified 2.7-fold from isolated envelopes. The enzyme could be stored at −70°C for several months with little loss in activity.

Subunit composition and molecular weight. An SDS-polyacrylamide gel electrophoresis profile of the peak fraction eluted from the DEAE-cellulose purification step is shown in Fig. 1. Two sharp bands with apparent molecular weights of 82,600 ± 1,700 (mean ± standard deviation, seven determinations) and 23,600 ± 300 and a broad band centered at 22,700 copurified with activity. The migration and intensity of the latter band were very dependent on protein concentration, detergent concentration, and the acrylamide/bisacrylamide ratio. Based on integration of densitometer scans of Coomassie blue-stained gels, the ratio of subunits was 1:1.1:1 (average of four experiments).

The native molecular weight of DMSO reductase was estimated by the gel electrophoresis method of Ferguson (12) to have a value of 155,000. This is in reasonable agreement with the predicted molecular weight of a holoenzyme composed of one copy of each of the three subunits observed in the SDS gel profiles (129,000). However, the possibility of multiple copies of the smaller subunits being associated with the holoenzyme cannot be eliminated. Interestingly, two protein bands were observed on the native gels of purified DMSO reductase (data not shown). These bands had identical slopes when the $\log_{10}$ of relative migration was plotted versus gel concentration, indicating that the two forms were of identical size although they differed in charge. Attempts to estimate the native molecular weight by gel exclusion chromatography resulted in an estimated size of 550,000. This rather high molecular weight may be due to anomolous migration of a detergent-protein complex or may result from aggregation of the enzyme.

TABLE 1. Purification of DMSO reductase

Fraction	Total protein (mg)	Total activity (U)[a]	Sp act (U/mg)	Yield (%)
Cell envelopes	816	5,520	6.76	100
Triton X-100 extract	485	6,880	14.2	125
Chromatofocusing eluate	243	4,090	16.8	74
DEAE-cellulose eluate	167	3,020	18.1	55

[a] Activity results were determined with DMSO as substrate.

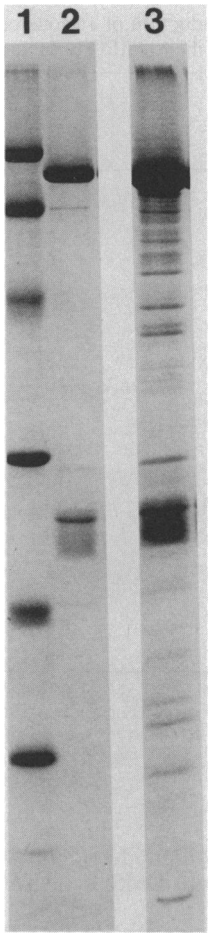

FIG. 1. SDS-polyacrylamide gel electrophoresis of enzyme fractions from chromatofocusing and DEAE-cellulose purification steps. Representative peak activity fractions were analyzed on a 12 to 17% SDS-polyacrylamide gradient gel and stained for protein with Coomassie blue. Lane 1, Molecular weight standards: phosphorylase *b*, 97,400; bovine serum albumin, 66,000; egg albumin, 45,000; carbonic anhydrase, 29,000; trypsin inhibitor, 20,100; and α-lactalbumin, 14,200. Lane 2, Peak activity fraction eluted from DEAE-cellulose with histidine buffer containing 100 mM KCl and 0.1% Triton X-100; 30 μg of protein was analyzed. Lane 3, Amicon-concentrated peak activity fraction from chromatofocusing purification step; 85 μg of protein was analyzed.

Substrate specificity. The purified reductase had a very broad substrate specificity. It utilized a number of sulfoxides and *N*-oxide compounds in addition to DMSO and TMAO (Table 2). The enzyme also reduced chlorate and hydroxylamine. The enzyme displayed simple Michaelis-Menten kinetics with all substrates examined except nicotinamide *N*-oxide, and the apparent K_m constants for several substrates are shown in Table 2. Nicotinamide *N*-oxide displayed substrate inhibition at high concentrations. In this case only the linear region of the Lineweaver-Burke plots was used for K_m determination. The K_m of 0.18 mM for DMSO, determined with the purified enzyme, agrees with the previously reported value of 0.17 mM, obtained with a crude membrane preparation (4). It was not possible to measure the K_m parameters for several of the *N*-oxides

TABLE 2. Reduction of various compounds by purified DMSO reductase

Substrate	Enzyme activity		K_m (mM)
	Substrate concn (mM)	Relative rate (%)[a]	
TMAO	10	100	0.6
Nicotinic acid *N*-oxide	10	109	—[b]
Nicotinamide *N*-oxide	3.4	134	0.5
2-Picoline *N*-oxide	10	84	—
3-Picoline *N*-oxide	10	124	—
4-Picoline *N*-oxide	10	134	1.0
Adenosine N^1-oxide	3.4	9	—
3-Hydroxypyridine *N*-oxide	10	99	—
Pyridine *N*-oxide	10	64	—
Picolinic acid *N*-oxide	5	<1	—
Hydroxylamine hydrochloride	10	25	—
DMSO	10	30	0.18
L-Methionine sulfoxide	10	28	0.47
Tetramethylene sulfoxide	10	34	—
Methyl phenyl sulfoxide	10	41	—
Phenyl sulfoxide	5	<1	—
Sodium chlorate	10	78	7.75
Sodium fumarate	10	<1	—
Sodium nitrate	10	<1	—
Sodium carbonate	10	<1	—

[a] 100% activity corresponds to 151.7 U of reductase activity per ml with TMAO as substrate. For measurement of the adenosine N^1-oxide or nicotinamide *N*-oxide activities, the substrate was dissolved directly in the assay buffer.

[b] —, Not determined.

because of their very limited solubility. The turnover numbers of the enzyme with DMSO and TMAO were 3,000 and 9,100, respectively.

Molybdopterin cofactor identification. We have previously shown that anaerobic growth of *E. coli* on DMSO was inhibited by the presence of sodium tungstate in the medium and that the *chl* genes, required for Mo cofactor assembly, were essential for growth on DMSO (4). These studies suggested that a molybdenum cofactor was essential for functional enzyme. To identify the cofactor in the enzyme, the oxidized form of the cofactor was isolated as described by Johnson and Rajagopalan (18). The UV absorption and fluorescence emission spectra of the cofactor (Fig. 2A and B, respectively) resembled those published for form A of a molybdopterin cofactor from sulfite oxidase (17). The crude cofactor had absorption maxima at 283 and 357 nm and a fluorescence emission maximum at 443 nm when excited at its excitation maximum of 357 nm.

To confirm the presence of the molybdopterin cofactor, reconstitution studies were carried out with the cofactor-deficient form of NADPH-nitrate reductase isolated from the *nit-1* mutant of *N. crassa* (15, 30). A crude molybdenum cofactor extract from DMSO reductase, with added molybdate, was able to reconstitute nitrate reductase activity in *N. crassa nit-1* extracts to approximately the same extent as demonstrated with the cofactor isolated from xanthine oxidase. No NADPH-nitrate reductase activity was detected when either *nit-1* or enzyme extracts were excluded from the reconstitution mixture.

Spectral properties. Purified DMSO reductase is visibly brownish-green in color. The enzyme had a typical UV absorption spectrum except for a shoulder at 290 nm presumably contributed by the molybdopterin cofactor (data not shown). The λ_{max} was 278 nm and an A_{280}/A_{260} ratio of 1.4 was observed. The A_{280} per milligram of protein was 1.0. The visible spectrum of the oxidized enzyme had a broad and rather nondescript absorption spectrum somewhat characteristic of iron-sulfur proteins. A fraction of this absorption was lost upon reduction with dithionite. The presence of iron-sulfur and molybdenum centers in the enzyme has been confirmed by electron paramagnetic resonance spectroscopy (R. Cammack, unpublished observations). No absorption bands or fluorescence signals characteristic of flavins were detected.

Metal analysis. The purified DMSO reductase was assayed by atomic emission spectroscopy for the presence of various metal atoms. The enzyme contained 7.5 atoms of iron per mol of holoenzyme. This agrees with the presence of iron-sulfur components in this enzyme. Only 0.34 atom of molybdenum was measured per mol of enzyme. This low value may result from the loss of metal from the molybdopterin cofactor and may also indicate that the purified enzyme is metal deficient. Copper, nickel, magnesium, and zinc were not detected in the purified enzyme.

Metal dependence. Attempts to stimulate enzyme activity by incubation with exogenous ammonium molybdate were unsuccessful (data not shown). A number of other metal ions (Co^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Mn^{2+}, Se^{4+}, and Zn^{2+}) were tested for their ability to stimulate DMSO reductase activity by their addition to the assay mixture. Of these, only Fe^{2+} stimulated DMSO reductase activity. A threefold increase in enzyme activity was observed by the addition of 300 μM $FeSO_4 \cdot 7H_2O$ to the standard assay mixture. It was previously reported that Mn^{2+}, Fe^{2+}, and Fe^{3+} were able to stimulate the activity of a purified amine *N*-oxide reductase of *E. coli* (29).

DISCUSSION

In this paper we describe the purification of a new membrane-bound terminal reductase of *E. coli*. To purify the enzyme to homogeneity, we made use of a recombinant DNA plasmid carrying the entire DMSO reductase operon. Anaerobically grown cells harboring this plasmid amplify DMSO reductase activity eight to ninefold, and only a threefold purification was needed to obtain a nearly homogeneous enzyme from the crude envelope fraction. Thus, the enzyme may account for up to 30% of the membrane protein in amplified strains. However, the 30% value was not reflected in the protein profiles of Coomassie-stained sam-

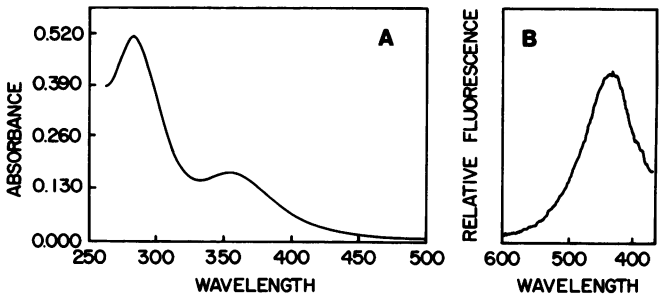

FIG. 2. UV absorption and fluorescence emission spectra of the molybdenum cofactor extracted from DMSO reductase. The cofactor was released from purified DMSO reductase by boiling the enzyme in the presence of KI and I_2 as described in Materials and Methods. (A) UV absorption spectrum of extracts in 50 mM sodium phosphate buffer, pH 6.8. (B) Fluorescence spectrum of the extracted cofactor. The excitation wavelength was 357 nm, and relative fluorescence was measured between 380 and 600 nm in 50 mM sodium phosphate buffer, pH 6.8.

ples analyzed on SDS-polyacrylamide gels (6). One possible explanation for this observation is the presence of other reductases in the cell envelope capable of reducing DMSO and therefore resulting in a high-specific-activity value for the cell envelope fraction. An inducible TMAO reductase in *E. coli* has been reported to reduce DMSO (36).

DMSO reductase is composed of three heterologous subunits present in equimolar ratios. The three polypeptides associated with this enzyme have also been identified from DNA sequence analysis of the chromosomal insert of pDMS159 (P. T. Bilous, S. T. Cole, W. F. Anderson, and J. H. Weiner, manuscript in preparation). Analysis of the polypeptides encoded by the open reading frames revealed the presence of a very hydrophobic polypeptide with a calculated molecular weight of 30,789. This subunit presumably provides the anchor domain for the two hydrophilic subunits of molecular weights 87,350 and 23,070. It is unclear which of the two smaller subunits identified on SDS-polyacrylamide gels represents this polypeptide. It is presumed to be the diffuse band with an apparent molecular weight of 22,700. Anomalous migration of this very hydrophobic polypeptide is not unexpected. A spuriously low M_r for *lac* permease, a hydrophobic membrane protein, has been reported (19).

The orientation of the enzyme in the membrane is not yet known; however, Bragg and co-workers have shown that anaerobic growth on DMSO or TMAO results in the induction of a periplasmic type *c* cytochrome which participates in electron transport to the reductase (8). This would suggest that the enzyme is at least partially exposed to the periplasm.

The purified enzyme exhibits a broad substrate specificity for *N*-oxides similar to that reported for TMAO reductases of *E. coli* (29, 36). In addition, the enzyme is capable of reducing several sulfoxides, with the lowest apparent K_m demonstrated for DMSO. In vivo metabolic competition experiments using ^{1}H-nuclear magnetic resonance have shown a preferential reduction of DMSO versus TMAO when challenged with both substrates, which would be expected from the K_m values determined for these substrates (P. T. Bilous, B. D. Sykes, and J. H. Weiner, unpublished observations). It is likely that both TMAO and DMSO serve as the true physiological substrates for DMSO reductase due to the widespread occurrence of these compounds in the natural environment (2, 3). It should be noted that DMSO reductase utilizes chlorate as a substrate, and along with nitrate reductase (16) and TMAO reductase (36), this enzyme may contribute to the chlorate sensitivity of *E. coli* cells. Interestingly, the enzyme used 3-hydroxypyridine *N*-oxide as a substrate. Various 2-hydroxypyridine *N*-oxide derivatives have been shown to be effective chelators of iron (20). It would be of interest to assay various 2-hydroxypyridine *N*-oxides as substrates for DMSO reductase and determine the effect of complexed iron on the activity.

There are now several reports indicating that DMSO and TMAO are reduced by a common terminal reductase. The results presented in this study are in agreement with studies in *Proteus vulgaris* (33) and *Rhodobacter capsulatus* (24) which suggest that one enzyme is responsible for both activities. The enzymatic and physical properties of several DMSO and TMAO reductases are summarized and compared in Table 3. DMSO reductase shares similar substrate specificity displayed by TMAO reductases from *E. coli* and other bacteria, but differs in subunit composition. In light of our findings, the presence of additional subunits associated with the other reductases should be investigated. We have designated DMSO reductase as a "constitutive" enzyme as it does not require the presence of exogenous DMSO or TMAO in the growth medium for anaerobic enzyme expression. However, as the enzyme displayed a very broad substrate specificity for *S*- and *N*-oxide compounds, other components in the growth medium or by-products of cell metabolism may induce its synthesis. It is not clear why *E. coli* should synthesize a number of enzymes which display similar substrate specificities (Table 3). A study of the conditions which induce and regulate the synthesis of these enzymes is necessary to understand their true physiological roles.

The sulfoxide tetrahydrothiophene oxide (tetramethylene sulfoxide) was recently reported to serve as electron acceptor for the anaerobic growth of *E. coli* (25). The substrate was reduced by a membrane-bound enzyme in a process requiring menaquinone and a molybdenum cofactor. Our data show that DMSO reductase can reduce tetrahydrothiophene oxide at a rate comparable to that found with DMSO. The broad substrate specificity shown in Table 2 probably explains the ability of *E. coli* to grow anaerobically with

TABLE 3. Properties of purified DMSO and TMAO reductases from various bacteria

Reductase	Source	Description[a]	Native mol wt[b]	Subunit mol wt[c]	K_m (mM)				pI	Reference
					DMSO	TMAO	MetSO	Chlorate		
DMSO	*E. coli*	Constitutive	155,000 (PAGE)	82,600 23,600 22,700	0.18	0.60	0.47	7.7	4.35	This study
TMAO	*E. coli*	Constitutive	160,000 (gel filtration)	NR[d]	NR	0.08	—[e]	NR	4.6	29
TMAO	*E. coli*	Inducible	200,000 (gel filtration)	95,000	+[f]	0.95	NR	+	NR	36
TMAO	*Salmonella typhimurium*	Inducible	332,000 (gel filtration) 170,000 (PAGE)	84,000	NR	0.89	NR	2.2	4.28	21
DMSO-TMAO	*P. vulgaris*	Inducible	95,000 (PAGE)	NR	+	0.3	NR	+	4.6	33
TMAO	*Rhodobacter capsulatus*	Constitutive	NR	46,000	+	+	+	+	NR	24

[a] Constitutive indicates anaerobic expression of the enzymne in cells grown in the absence of substrate. Inducible enzyme requires the presence of substrate (DMSO or TMAO) for anaerobic expression.
[b] Native molecular weights were determined either by gel filtration or nondenaturing polyacrylamide gel electrophoresis (PAGE) as indicated.
[c] Subunit molecular weights were determined by SDS-polyacrylamide gel electrophoresis.
[d] NR, Not reported.
[e] —, No activity.
[f] +, Activity present but not quantitated.

several of these substrates as terminal electron acceptors, and it is not necessary to invoke a series of distinct enzymes.

All molybdoenzymes, with the exception of nitrogenase, contain a common molybdenum cofactor which consists of Mo complexed with a novel pterin molecule (27). Several *E. coli* enzymes, such as nitrate reductase (16), formate dehydrogenase (16), TMAO reductase (36), and biotin sulfoxide reductase (10), have been identified as molybdoenzymes. Our initial studies on DMSO reduction indicated a requirement for the cofactor (4). Analyses of the purified DMSO reductase clearly establish that this enzyme contains molybdenum and a typical molybdopterin cofactor. Work is presently in progress to identify the cofactor-binding domain in the enzyme.

ACKNOWLEDGMENTS

This work was funded by a grant (MT3858) from the Medical Research Council of Canada. P.T.B. is a postdoctoral fellow of the Alberta Heritage Foundation for Medical Research, from which R.E.B. received a summer studentship.

We thank Cecylia Bilous (Dow Chemical Ltd.) for carrying out the atomic emission measurements.

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