Asymmetric reduction of racemic sulfoxides by dimethyl sulfoxide reductases from *Rhodobacter capsulatus*, *Escherichia coli* and *Proteus* species

Steven P. Hanlon,^{1†} Daniel L. Graham,^{2‡} Philip J. Hogan,³
Robert A. Holt,² Christopher D. Reeve,² Anthony L. Shaw⁴
and Alastair G. McEwan⁴

Author for correspondence: Alastair G. McEwan. Tel: +61 7 3365 4878. Fax: +61 7 3365 4620.
e-mail: mcewan@biosci.uq.edu.au

¹ School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, UK

² Zeneca LifeScience Molecules, Belasis Avenue, Billingham, Cleveland TS23 1YN, UK.

³ Zeneca Pharmaceuticals, Macclesfield, Cheshire, SK10 2NA, UK

⁴ Department of Microbiology, The University of Queensland, Brisbane 4072, Australia

The enantioselective reduction of racemic sulfoxides by dimethyl sulfoxide reductases from *Rhodobacter capsulatus*, *Escherichia coli*, *Proteus mirabilis* and *Proteus vulgaris* was investigated. Purified dimethyl sulfoxide reductase from *Rhodobacter capsulatus* catalysed the selective removal of (S)-methyl *p*-tolyl sulfoxide from a racemic mixture of methyl *p*-tolyl sulfoxide and resulted in an 88% recovery of enantiomerically pure (R)-methyl *p*-tolyl sulfoxide. *Rhodobacter capsulatus* was shown to be able to grow photoheterotrophically in the presence of certain chiral sulfoxides under conditions where a sulfoxide is needed as an electron sink. Whole cells of *Rhodobacter capsulatus* were shown to catalyse the enantioselective reduction of methyl *p*-tolyl sulfoxide, ethyl 2-pyridyl sulfoxide, methylthiomethyl methyl sulfoxide and methoxymethyl phenyl sulfoxide. Similarly, whole cells of *Escherichia coli*, *Proteus mirabilis* and *Proteus vulgaris* reduced these sulfoxides but with opposite enantioselectivity.

Keywords: dimethyl sulfoxide reductases, racemic sulfoxides, enantioselectivity

INTRODUCTION

The importance of chirality to the specificity of drug action is well documented. For example, the activity of the (S)-enantiomer of the β -receptor antagonist propranolol is approximately 100-fold that of the (R)-enantiomer (Bevinakatti & Banerji, 1991; Crosby, 1992). A valuable chemical group which can be used in chiral drug synthesis is the sulfoxide. When substituted with two different groups the sulfoxide is chiral by virtue of the presence of a lone pair of electrons on the sulfur atom. Optically active sulfoxides have been used as functional groups in drugs (see Pitchen *et al.*, 1994, for example) and also as reagents for the control of

diastereoselective reactions at adjacent substituents (Carreno, 1995; Butlin *et al.*, 1996). The development of methods for the production of optically pure compounds is a rapidly expanding industry in which enzymes play a central role (Roberts *et al.*, 1995). Although a number of chemical methods for the production of optically active sulfoxides have been investigated (Solladie, 1981; Carreno, 1995) biological approaches have also been applied. For example, racemic sulfoxides containing an ester group have been resolved using ester hydrolase (Burgess *et al.*, 1992) and prochiral sulfides have been asymmetrically oxidized by both whole cells (Rossi *et al.*, 1992; Holland, 1988; Holland *et al.*, 1995) and isolated enzymes (Colonna *et al.*, 1990).

An alternative approach to the preparation of optically active sulfoxides is the enantioselective reduction of racemic sulfoxides by S-oxide reductases. Bacterial S-oxide reductases are widely distributed among facultative aerobic bacteria, where they function as the terminal enzyme in an anaerobic respiratory chain (McEwan *et al.*, 1991; Weiner *et al.*, 1992). The dimethyl sulfoxide (DMSO) reductase (DmsABC) from *Escher-*

[†] Present address: Molecular Pharmacology Unit, Ninewells Hospital and Medical School, Dundee DD1 9SY, UK.

[‡] Present address: Department of Biochemistry, University College, Gower Street, London WC1E 6BT, UK.

Abbreviations: DMSO, dimethyl sulfoxide; EPSO, ethyl 2-pyridyl sulfoxide; FMN, flavin mononucleotide; MMPSO, methoxymethyl phenyl sulfoxide; MPTSO, methyl *p*-tolyl sulfoxide; MTMSO, methylthiomethyl methyl sulfoxide; TMAO, trimethylamine-*N*-oxide.

ichia coli is a membrane-bound enzyme which catalyses electron transfer from menaquinol to a wide variety of *S*-oxides and *N*-oxides (Weiner *et al.*, 1988; Simala-Grant & Weiner, 1996). The enzyme is composed of three subunits: a catalytic subunit (DmsA) which contains a pterin molybdenum cofactor, a subunit which contains four [4Fe-4S] clusters (DmsB) and a membrane-bound anchor subunit (DmsC) (Weiner *et al.*, 1992). The second type of DMSO reductase is a periplasmic enzyme which terminates a respiratory chain in purple phototrophic bacteria such as *Rhodobacter capsulatus* (McEwan *et al.*, 1985) and *R. sphaeroides* (Satoh & Kurihara, 1987). This enzyme also contains a pterin molybdenum cofactor (McEwan *et al.*, 1991), and high-resolution X-ray crystallographic structures of this DMSO reductase have been reported (Schindelin *et al.*, 1996; Schneider *et al.*, 1996; McAlpine *et al.*, 1997).

Sequence analysis of the *R. capsulatus* DMSO reductase has shown that it is more closely related to the periplasmic trimethylamine-*N*-oxide (TMAO) reductase (TorA) from *E. coli* rather than DmsA (Shaw *et al.*, 1996) and for this reason we refer to the *Rhodobacter* DMSO reductase as DorA. In contrast to the *E. coli* TorA, which exhibits a high k_{cat}/K_m ratio with only a few *N*-oxides (Iobbi-Nivol *et al.*, 1996), DorA has a relatively broad substrate specificity towards *S*-oxides and *N*-oxides (Satoh & Kurihara, 1987). It has been established that DorA is enantioselective when reacting with chiral sulfoxides (Hanlon, 1993; Abo *et al.*, 1994, 1995) but nothing is known about the enantioselectivity of DmsABC. In this paper we describe the reaction of chiral sulfoxides with purified DorA from *R. capsulatus* and the enantioselective reduction of chiral sulfoxides by cells of *R. capsulatus*, *E. coli* and *Proteus* species.

METHODS

Chemicals. The structures of the *S*-oxides used in this study are shown in Fig. 1. Hydrogen peroxide, *m*-chloroperbenzoic acid, sodium periodate, methyl *p*-tolyl sulfoxide (MPTSO) (pure enantiomers), methyl *p*-tolyl sulfide, methoxymethyl phenyl sulfide, methylthiomethyl methyl sulfoxide (MTMSO), bis(methylthio)methane, *tert*-butyl methyl sulfide, DL-methionine sulfoxide, dimethyl sulfoxide, 2-mercaptopyridine and ethyl bromide were obtained from Aldrich. Sulfides and sulfoxides not commercially available were synthesized as follows.

***tert*-Butyl methyl sulfoxide.** *tert*-Butyl methyl sulfide (1.5 g) was added to glacial acetic acid (50 ml) at room temperature and stirred. An equimolar equivalent of hydrogen peroxide (30 wt% in water) was added over a period of 4 h whilst maintaining the temperature below 20 °C. After a further 1 h the reaction was diluted with water (200 ml) and then extracted with dichloromethane (250 ml). The organic layer was recovered and washed successively with equal volumes of water, 0.5 M sodium hydrogen carbonate and water. The organic layer was dried with anhydrous sodium sulfate and the solvent removed under reduced pressure to yield *tert*-butyl methyl sulfoxide as a clear oil.

Methoxymethyl phenyl sulfoxide (MMPSO). Methoxymethyl phenyl sulfide (5.1 g) was added to dichloromethane (100 ml) and stirred on ice. *m*-Chloroperbenzoic acid (7.7 g) was dissolved

Fig. 1. Structures of *S*-oxides used in this study.

in dichloromethane (120 ml) and added dropwise to the sulfide solution over a period of 1 h. The reaction was stirred for 3 h then washed successively with aqueous 10% sodium sulfite (2 × 50 ml) and water (2 × 50 ml). The dichloromethane layer was dried with anhydrous sodium sulfate and evaporated under reduced pressure to yield a yellow oil (5.7 g). Residual sulfide was removed by purification on a 30 g silica gel column (Kieselgel 60, Merck) eluted with ethylacetate/hexane (1:1, v/v) to yield 1.3 g of a clear, odourless oil.

Ethyl 2-pyridyl sulfoxide (EPSO). To a solution of 2-mercaptopyridine (17.85 g) in *N*-methylpyrrolidone (250 ml) was added 49% aqueous potassium hydroxide (9 ml). Ethyl bromide (16.2 ml) was added over 7 min whilst maintaining the temperature below 30 °C (a white solid precipitated). The stirred reaction was heated to 85 °C for 3 h then allowed to cool to 20 °C over 15 h. Toluene (320 ml) and water (320 ml) were added to the mixture resulting in a temperature rise to 34 °C. The mixture was cooled to 20 °C and the aqueous phase adjusted to pH 11 by the dropwise addition of 49% potassium hydroxide (~6 ml). The organic and aqueous layers were separated and the aqueous phase (566 ml) re-extracted with toluene (160 ml). The organic phase was washed with 2 M sodium hydroxide (2 × 160 ml) and water (2 × 160 ml). The recovered organic phase was evaporated under reduced pressure to yield 23.04 g of a yellow oil. A sample of the oil (22.71 g) was transferred to a 50 ml flask and distilled at 80 mmHg pressure using a 5 cm Vigreux column; the product, ethyl 2-pyridyl sulfide, distilled at a head temperature of 124–125 °C. The sulfide (18.1 g) was mixed with methanol (26 ml) and added dropwise to an aqueous solution of sodium periodate (29.6 g in 278 ml) over 20 min whilst maintaining the temperature at 0–3 °C. The periodate addition funnel was washed with a further 26 ml of methanol, which was added to the reaction over 5 min. The reaction was held at 0 °C for 44 h, filtered, and the filter cake washed with water (63 ml). The combined filtrate was extracted with dichloromethane (3 × 160 ml) and the solvent evaporated under reduced pressure to give a colourless oil (19.5 g). The

crude sulfoxide was purified by fractional distillation under reduced pressure (4 mmHg, 131–132 °C).

Micro-organisms and growth. Bacteria used in this study were *Escherichia coli* ATCC 33694, *Rhodobacter capsulatus* DSM 938 (also known as 37b4), *R. capsulatus* H123 (Hudig *et al.*, 1986), *Proteus vulgaris* NCIMB 67 and *Proteus mirabilis* NCIMB 8268. For chemoheterotrophic growth micro-organisms were typically grown in a basal medium of the following composition (g l⁻¹): NaH₂PO₄, 1.56; K₂HPO₄, 1.9; (NH₄)₂SO₄, 1.8; MgSO₄·7H₂O, 0.2; FeCl₃, 0.005; Na₂MoO₄·2H₂O, 0.001; yeast extract, 2 or Casamino acids, 1. After autoclaving, the following components were added as a filter-sterilized solution (final concentrations g l⁻¹): thiamin·HCl, 0.03; nicotinic acid, 0.007; DMSO, 5.5. Trace elements solution (1 ml l⁻¹) was also added to the medium. The stock trace elements solution contained (g l⁻¹): CuSO₄·5H₂O, 0.02; MnSO₄·4H₂O, 0.1; ZnSO₄·7H₂O, 0.1; CaCO₃, 1.8. The carbon source for growth was either glucose or glycerol (5 or 10 g l⁻¹).

Bacteria were grown under essentially anaerobic conditions in 1 litre screw-capped conical flasks containing 800 ml medium and incubated at 30 °C for 48 h with gentle mixing on a rotary shaker. For phototrophic growth of *R. capsulatus*, RCV medium (Weaver *et al.*, 1975) supplemented with DL-malic acid (4 g l⁻¹) was used. For assessment of sulfoxides as respiratory electron acceptors under anaerobic conditions RCV medium without malate but supplemented with sodium propionate (30 mM) (RCV/propionate) and the appropriate sulfoxide (10 mM) was used. Cultures were grown in glass bottles between two banks of tungsten lights. Bottles were completely filled to ensure that anaerobic conditions were obtained.

Purification of DMSO reductase from *R. capsulatus*. DMSO reductase was purified from *R. capsulatus* H123, a derivative strain of DSM 938 (Hudig *et al.*, 1986), as described by McEwan *et al.* (1991) with the modifications of Bennett *et al.* (1994).

Enzyme assays. DMSO reductase activity was monitored spectrophotometrically using dithionite-reduced methyl viologen as electron donor as described by McEwan *et al.* (1985). Oxidation of reduced methyl viologen was monitored at 600 nm and an extinction coefficient of 13 mM⁻¹ (Jones & Garland, 1976) used to calculate reaction rates. Kinetic constants for DMSO reductase were determined by the use of Lineweaver–Burk plots.

For experiments to determine the enantioselectivity of purified DMSO reductase a photocatalysed flavin-linked reaction system was devised. A gas-tight glass syringe (5 ml) with a valve was filled with 3 ml anaerobic Tris/HCl (50 mM, pH 8.0) buffer containing EDTA (10 mM). To this was added FMN (250 µM final concentration) and racemic sulfoxide (5–20 mM final concentration). The syringe was illuminated using tungsten light (three 100 W bulbs placed 20 cm from the syringe), resulting in the reduction of FMN (Penzer & Radda, 1971). When FMN reduction was complete as judged by decolouration of the yellow solution the reaction was started by the introduction of a solution of DMSO reductase and the volume made up to 5 ml with buffer. Samples were taken at intervals and analysed by HPLC.

Whole-cell reactions with sulfoxides. Following growth, bacteria were recovered by centrifugation and washed in potassium phosphate buffer (100 mM, pH 7.6). The recovered cell pellets were then resuspended in fresh buffer of the same composition to a concentration of 1–5 g dry cell weight l⁻¹. In a typical reaction cell suspension (20 ml) was transferred to a

50 ml serum bottle containing racemic sulfoxide (40–60 mg); the bottle was sealed with a rubber stopper and the headspace flushed with nitrogen. Reaction mixtures were incubated at 35 °C without agitation and samples taken at intervals for analysis by HPLC.

Analysis of reactants and products. The concentrations of sulfides and sulfoxides were determined by either HPLC or GC.

MMP SO, EPSO and their corresponding sulfides were analysed on a Spherisorb S5 ODS2 HPLC column (250 × 4.6 mm) eluted at a flow rate of 1 ml min⁻¹ (eluant composition 50% acetonitrile, 50% sodium phosphate buffer, 25 mM, pH 7.6); detection was by UV absorption at 270 nm. EPSO and the corresponding sulfide eluted at 2.4 and 6.5 min respectively. MMP SO and the corresponding sulfide eluted at 2.6 and 8.3 min respectively. MPT SO and the corresponding sulfide were analysed under the same conditions except that the eluant flow rate was decreased to 0.8 ml min⁻¹ and the elution times were 5.0 and 18.0 min respectively. Samples were prepared for analysis by removal of cells by centrifugation followed by dilution of the supernatant in HPLC eluant.

MTMSO and the corresponding sulfide, bis(methylthio)-methane, were analysed by GC on a J&W Scientific DB5 column (28 m × 0.328 mm, film thickness, 1.0 µm) using helium as carrier gas and flame ionization detection. The temperature programme consisted of an initial isothermal period, 150 °C for 3 min, followed by an increase to 200 °C at a rate of 20 °C min⁻¹ and a final isothermal period of 2 min. Samples were prepared for analysis by removal of cells by centrifugation, then the supernatant was diluted with an equal volume of aqueous 2,3-butanediol (2 g l⁻¹), which acted as internal standard. MTMSO eluted at 4.9 min, the corresponding sulfide at 2.7 min and the internal standard at 2.2 min.

The optical purity of EPSO was determined using a Chiralcel OB HPLC column (250 × 4.6 mm) eluted at a rate of 0.8 ml min⁻¹ with hexane/2-propanol (85:15, v/v). Detection was by UV absorption at 270 nm. Ethyl 2-pyridyl sulfide eluted at 9.5 min whilst the enantiomers of EPSO eluted at 16.5 and 29.5 min (the absolute configuration of the two peaks was not assigned).

The optical purity of the other sulfoxides was determined using a Chiralcel OD HPLC column (250 × 4.6 mm) eluted at a rate of 0.8 ml min⁻¹ with hexane/ethanol (95:5, v/v). Detection was by UV absorption at 270 nm. Methoxymethyl phenyl sulfide eluted at 6.1 min whilst the enantiomers of MMP SO eluted at 24.3 and 28.7 min. Methyl *p*-tolyl sulfide eluted at 5.9 min; (R)- and (S)-MPT SO eluted at 15.5 and 17.1 min respectively. MTMSO enantiomers eluted at 31 and 32.5 min and were detected by UV absorption at 225 nm; bis(methylthio)methane was not detectable by UV absorption.

RESULTS AND DISCUSSION

Enantioselective reduction of racemic MPT SO by *R. capsulatus* DorA

R. capsulatus DorA has a high sequence similarity to TMAO reductase (TorA) from *E. coli* (45% sequence identity) (Shaw *et al.*, 1996) but the enzymes differ in their substrate specificity. While the former is able to use both *S*-oxides and *N*-oxides (McEwan *et al.*, 1985), the latter is highly specific for TMAO and does not use *S*-oxides at significant rates (Iobbi-Nivol *et al.*, 1996). Although *R. capsulatus* DorA has only 29% sequence

Fig. 2. Enantioselective reduction of racemic MPTSO by *R. capsulatus* DorA using photochemically reduced FMN as electron donor. ♦, (R)-MPTSO; ■, (S)-MPTSO; ▼, enantiomeric excess.

identity to *E. coli* DmsA (Shaw *et al.*, 1996) the two enzymes exhibit a broad substrate specificity. The kinetic parameters of DorA from *R. capsulatus* and *R. sphaeroides* show that while the k_{cat} values for the reduction of DMSO and TMAO are relatively close the K_m value for DMSO is in the micromolar range while the K_m for TMAO is in the millimolar range (Satoh & Kurihara, 1987; Benson, 1993). In view of the broad substrate specificity of DMSO reductases, we were interested to determine whether the *R. capsulatus* DorA could catalyse the enantioselective reduction of racemic sulfoxides. An initial experiment was performed in which the kinetic parameters for the reduction of the enantiomers of MPTSO were determined. Although the k_{cat} values for the enantiomers of MPTSO were similar the K_m value for (S)-MPTSO was about 100-fold lower than the K_m for (R)-MPTSO (data not shown). This observation was consistent with previous work by Abo *et al.* (1994), who showed that *R. sphaeroides* DorA

catalysed the stereoselective removal of (S)-methyl phenyl sulfoxide from a racemic mixture.

The observation that DorA had different kinetic parameters with the two enantiomers of MPTSO suggested that treatment of racemic MPTSO with *R. capsulatus* DorA would result in the relative enrichment of the (R)-enantiomer due to the more rapid reduction of the (S)-enantiomer. This was tested using purified DorA in a photochemically driven reaction system with FMNH₂ as electron donor. Samples were removed from the reaction at intervals and analysed by HPLC to determine the extent of reduction and the ratio of enantiomers. Fig. 2 shows that (S)-MPTSO decreased far more rapidly with time than the (R)-MPTSO; this is consistent with the kinetic results using pure enantiomers. The (R)-MPTSO was produced with an enantiomeric excess of 100%, with an 88% recovery of this enantiomer.

Anaerobic phototrophic growth of *R. capsulatus* using various sulfoxides as auxiliary oxidants

DMSO respiration is one of several anaerobic respiratory pathways which have been identified in *Rhodospirillum rubrum* (reviewed by McEwan, 1994). The use of electron acceptors such as DMSO is not essential for anaerobic phototrophic growth but their reduction can facilitate anaerobic growth on reduced carbon sources such as propionate or butyrate (Richardson *et al.*, 1988). As a consequence, these electron acceptors have been described as auxiliary oxidants (McEwan *et al.*, 1985). The requirement of an auxiliary oxidant for growth on propionate opened the way for screening a range of sulfoxides as potential substrates for DorA. A series of growth curves was obtained for the phototrophic growth of *R. capsulatus* DSM 938 on RCV/propionate medium. In the absence of auxiliary oxidants no growth was observed whilst in the presence of DMSO the doubling time was 4 h. Four sulfoxides tested supported anaerobic growth, albeit with longer doubling times than observed with DMSO. These were MPTSO (t_d 12 h), MPTSO (t_d 12 h), methionine sulfoxide (t_d 7.5 h)

Table 1. Enantioselective reduction of MPTSO by whole cells of *R. capsulatus*, *E. coli* and *P. mirabilis*

R. capsulatus was grown phototrophically on RCV medium supplemented with yeast extract (2 g l⁻¹) and glucose (10 g l⁻¹). Enteric bacteria were grown on a basal medium supplemented with Casamino acids (1.5 g l⁻¹), plus glucose (5 g l⁻¹) for *P. mirabilis* and glycerol (5 g l⁻¹) for *E. coli*. Concentrations of MPTSO enantiomers are given in mg ml⁻¹. —, No measurement made.

Time (h)	<i>E. coli</i>		<i>P. mirabilis</i>		<i>R. capsulatus</i>	
	(R)-MPTSO	(S)-MPTSO	(R)-MPTSO	(S)-MPTSO	(R)-MPTSO	(S)-MPTSO
0	0.74	0.74	0.74	0.74	0.25	0.25
0.25	—	—	—	—	0.20	0
4	0.27	0.63	0.08	0.72	—	—
20	0	0.45	0	0.58	—	—

Fig. 3. Enantioselective reduction of sulfoxides by whole cells of *R. capsulatus* (a), *E. coli* (b) and *P. vulgaris* (c). The enantiomeric ratio refers to proportion of one enantiomer as a percentage of the total racemic mixture as determined by chiral HPLC, under starting conditions where each enantiomer represented 50% of the mixture. The figure shows the decrease in the 28.7 min, 32.5 min and 16.5 min elution peaks of one enantiomer of MMPSO (◇), MTMSO (▽) and EPSO (○) respectively. (a) *R. capsulatus* was grown phototrophically in RCV medium supplemented with yeast extract (2 g l⁻¹) and glucose (10 g l⁻¹). Concentrations of sulfoxides at the beginning of the experiment were: EPSO, 3.2 mg ml⁻¹; MMPSO, 2.3 mg ml⁻¹; and MTMSO, 2.4 mg ml⁻¹. (b, c) *E. coli* and *P. vulgaris* were grown on a basal medium supplemented with glucose (10 g l⁻¹) and yeast extract (2 g l⁻¹). Concentrations of sulfoxides at the beginning of the experiment were: EPSO, 2.6 mg ml⁻¹; MMPSO, 2.5 mg ml⁻¹; and MTMSO, 2.4 mg ml⁻¹.

and *tert*-butyl methyl sulfoxide (doubling time not determined).

Although racemic MPTSO supported growth with a doubling time of 12 h no growth was observed over a period of 45 h when (*R*)-MPTSO was supplied as auxiliary oxidant. This is consistent with the demonstrated enantioselectivity of DorA from *R. capsulatus*. Similarly, Simala-Grant & Weiner (1996) have shown that a variety of *N*-oxides and *S*-oxides can support anaerobic growth of *E. coli* and this must involve the use of DmsABC in respiration.

Resolution of racemic sulfoxides by whole cells of *R. capsulatus*, *E. coli* and *Proteus* sp.

The enantioselective reduction of MPTSO by DorA from *R. capsulatus* prompted us to investigate the enantioselectivity of other bacterial *S*-oxide reductases. *E. coli*, *P. mirabilis* and *P. vulgaris* were selected for investigation and compared with *R. capsulatus* DSM 938 using a range of sulfoxide substrates. It was already known that *E. coli* DmsABC could reduce a number of racemic sulfoxides and *N*-oxides (Simala-Grant & Weiner, 1996) but nothing was known about its enantioselectivity. In order to avoid the need to purify the enzymes these reactions were carried out using whole cells. All of the bacteria were able to reduce the four racemic sulfoxides (MPTSO, MTMSO, MMPSO and EPSO) in an enantioselective way. As expected, *R. capsulatus* used the (*S*)-MPTSO enantiomer in preference to the (*R*)-enantiomer (Table 1) but surprisingly it was observed that *E. coli* carried out a reaction which was enantiocomplementary and resulted in the preferential removal of the (*R*)-MPTSO (Table 1). In view of the latter result a second enteric bacterium, *P. mirabilis*, was investigated to determine whether its enantioselectivity was similar to that of *R. capsulatus* or *E. coli* (Table 1). It was found that, like *E. coli*, *P. mirabilis* preferentially removed (*R*)-MPTSO (Table 1).

Further experiments to investigate the resolution of the three other racemic sulfoxides showed that the enantioselectivity of *R. capsulatus* was opposite to that of *E. coli* and *P. vulgaris*. Although the absolute configuration of the enantiomers was not determined they were characterized by their distinct elution times during resolution by chiral HPLC. Fig. 3(a) shows that with *R. capsulatus* for EPSO, MMPSO and MTMSO there was a decrease in the percentage of one enantiomer in each racemic mixture. Thus, in the case of EPSO, one enantiomer (16.5 min elution peak) within the mixture began as 50% of the total but decreased until it formed almost a zero proportion of the mixture. A similar result was obtained for MTMSO (decrease in the 32.5 min elution peak) while in the case of MMPSO (decrease in the 28.7 min elution peak) the percentage of this racemate within the mixture was 30% at the end of the experiment. Although the configuration of the enantiomers which were removed was not established it seems likely, in view of the results with MPTSO, that it was the (*S*)-racemate which was removed. In contrast, Fig. 3(b)

shows that for *E. coli* the same enantiomers within each racemic mixture which decreased most rapidly in racemic mixtures treated with *R. capsulatus* were observed to increase as a percentage of the total. This indicates that the *E. coli* cells were removing the opposite enantiomer of each sulfoxide compared to *R. capsulatus*. The results for the *P. vulgaris* cells (Fig. 3c) were similar to those with *E. coli*. Taken together, these data show that the enantioselectivity of *R. capsulatus* chiral sulfoxides is opposite to that of *E. coli* and *P. vulgaris*. Since it is established that *E. coli* TMAO reductase is unable to reduce *S*-oxides while DmsABC has broad specificity towards these substrates, including MPTSO (Simala-Grant & Weiner, 1996), it seems highly likely that this enzyme was responsible for the enantioselective reduction of these racemic sulfoxides. However, we cannot rule out the possibility that other cytoplasmic *S*-oxide reductases, such as biotin sulfoxide reductase, which have been identified in the *E. coli* genome (Blattner *et al.*, 1997) made a minor contribution to the reduction of the racemic sulfoxides. Results using *P. vulgaris* and *P. mirabilis* were broadly similar to those for *E. coli* (Table 1, Fig. 3), suggesting that the properties of the *Proteus* *S*-oxide/*N*-oxide reductase was closely related to that of the *E. coli* DmsABC. This is consistent with previous studies of DMSO reductase in *P. vulgaris* which characterized a membrane-bound enzyme which was able to reduce both DMSO and TMAO (Styrvoid & Strom, 1984). It is notable that in all three systems both alkylaryl and dialkyl sulfoxides were resolved. Although we did not investigate diaryl sulfoxide reduction, Simala-Grant & Weiner (1996) have shown that diphenyl sulfoxide is reduced at an extremely low rate by DmsABC of *E. coli*.

Concluding remarks

In this paper we have shown that DMSO reductases from purple phototrophic bacteria and enteric bacteria catalyse the enantioselective reduction of racemic sulfoxides with opposite stereospecificity. The determination of the structure of DorA with DMSO bound (McAlpine *et al.*, 1998) opens the way for molecular modelling of the binding of chiral sulfoxides to this enzyme; this work should provide a deeper insight into the molecular basis of enantioselectivity in *R. capsulatus* DorA. Further comparisons with DmsA will also be informative in gaining an understanding of the molecular basis for the difference in enantioselectivity between DorA and DmsABC. It will also be interesting to investigate the enantioselectivity of DMSO reductases towards chiral *N*-oxides.

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REFERENCES

- Abo, M., Tachibana, M., Okubo, A. & Yamazaki, S. (1994). Enantioselective reduction of the sulfoxide to sulfide in methyl phenyl sulfoxide by dimethylsulfoxide reductase from *Rhodobacter sphaeroides* f.s. *denitrificans*. *Biosci Biotechnol Biochem* **58**, 596–597.
- Abo, M., Tachibana, M., Okubo, A. & Yamazaki, S. (1995). Enantioselective deoxygenation of alkyl aryl sulfoxides by DMSO reductase from *Rhodobacter sphaeroides* f.s. *denitrificans*. *Bioorg Med Chem* **3**, 109–112.
- Bennett, B., Benson, N., McEwan, A. G. & Bray, R. C. (1994). Multiple states of the molybdenum centre of dimethylsulfoxide reductase from *Rhodobacter capsulatus* revealed by EPR spectroscopy. *Eur J Biochem* **225**, 321–331.
- Benson, N. (1993). *Biochemical and spectroscopic studies of dimethylsulphoxide reductase from Rhodobacter capsulatus*. PhD thesis, University of East Anglia.
- Bevinakatti, H. S. & Banerji, A. A. (1991). Practical chemo-enzymatic synthesis of both enantiomers of propanolol. *J Org Chem* **56**, 5372–5375.
- Blattner, F. R., Plunkett, G., 3rd, Bloch, C. A. & 14 other authors (1997). The complete genome sequence of *Escherichia coli* K-12. *Science* **277**, 1453–1474.
- Burgess, K., Henderson, I. & Ho, K.-K. (1992). Biocatalytic resolutions of sulfinylalkanoates: a facile route to optically active sulfoxides. *J Org Chem* **57**, 1290–1295.
- Butlin, R. J., Linney, I. D., Mahon, M. F., Tye, H. & Wills, M. (1996). Recoverable chiral sulfoxides for asymmetric synthesis: application to stereoselective carbonyl reduction and the asymmetric synthesis of allylic alcohols. *J Chem Soc Perkins Trans* **1**, 95–105.
- Carreno, M. C. (1995). Applications of sulfoxides to asymmetric synthesis of biologically active compounds. *Chem Rev* **95**, 1717–1760.
- Colonna, S., Gaggero, N., Manfredi, A., Casella, L., Gullotti, M., Carrea, G. & Pasta, P. (1990). Enantioselective oxidations of sulfides catalysed by chloroperoxidase. *Biochemistry* **29**, 10465–10468.
- Crosby, J. (1992). Chirality in industry – an overview. In *Chirality in Industry*, vol. 1, pp. 1–66. Edited by A. N. Collins, G. N. Sheldrake & J. Crosby. Chichester: Wiley.
- Hanlon, S. P. (1993). *Physiology and biochemistry of dimethylsulfide oxidation and dimethylsulfoxide reduction by Rhodobacter species*. PhD thesis, University of East Anglia.
- Holland, H. L. (1988). Chiral sulfoxidation by biotransformation of organic sulfides. *Chem Rev* **88**, 473–485.
- Holland, H. L., Brown, F. M. & Larsen, B. G. (1995). Biotransformation of organic sulfides. 6. Formation of chiral para substituted benzyl methyl sulfoxides by *Helminthosporium* species NRRL 4671. *Tetrahedron Asymmetry* **6**, 1561–1567.
- Hudig, H., Kaufmann, N. & Drews, G. (1986). Respiratory deficient mutants of *Rhodopseudomonas capsulata*. *Arch Microbiol* **145**, 378–385.
- Iobbi-Nivol, C., Pommier, J., Simala-Grant, J., Mejean, V. & Giordano, G. (1996). High substrate specificity and induction characteristics of trimethylamine-*N*-oxide reductase of *Escherichia coli*. *Biochim Biophys Acta* **1294**, 77–82.
- Jones, R. W. & Garland, P. B. (1976). Sites and specificity of the reaction of bipyridylum compounds with anaerobic respiratory enzymes of *Escherichia coli*. Effects of permeability barriers imposed by the cytoplasmic membrane. *Biochem J* **164**, 199–211.

- McAlpine, A. S., McEwan, A. G., Shaw, A. L. & Bailey, S. (1997). Molybdenum active centre of DMSO reductase from *Rhodobacter capsulatus*: crystal structure of the oxidised enzyme at 1.82 Å resolution and the dithionite-reduced enzyme at 2.8 Å resolution. *J Biol Inorg Chem* 2, 690–701.
- McAlpine, A. S., McEwan, A. G. & Bailey, S. (1998). The high resolution crystal structure of DMSO reductase in complex with DMSO. *J Mol Biol* 275, 613–623.
- McEwan, A. G. (1994). Photosynthetic electron transport and anaerobic metabolism in purple non-sulfur phototrophic bacteria. *Antonie Leeuwenhoek* 66, 151–164.
- McEwan, A. G., Wetzstein, H.-G., Jackson, J. B. & Ferguson, S. J. (1985). Periplasmic location of the terminal reductase in trimethylamine-N-oxide and dimethylsulfoxide respiration in the photosynthetic bacterium *Rhodospseudomonas capsulata*. *Biochim Biophys Acta* 806, 410–417.
- McEwan, A. G., Ferguson, S. J. & Jackson, J. B. (1991). Purification and properties of dimethylsulphoxide reductase from *Rhodobacter capsulatus*. *Biochem J* 207, 305–307.
- Penzer, G. R. & Radda, G. K. (1971). Photochemistry of flavins. *Methods Enzymol* 18B, 479–495.
- Pitchen, P., France, C. J., McFarlane, I. M., Newton, C. G. & Thompson, D. M. (1994). Large scale synthesis of a biologically active sulfoxide. *Tetrahedron Lett* 35, 485–488.
- Richardson, D. J., King, G. F., Kelly, D. J., McEwan, A. G., Jackson, J. B. & Ferguson, S. J. (1988). The role of auxiliary oxidants in maintaining redox balance during phototrophic growth of *Rhodobacter capsulatus* on propionate and butyrate. *Arch Microbiol* 150, 130–137.
- Roberts, S. M., Turner, N. J., Willets, A. J. & Turner, M. K. (1995). *Introduction to Biocatalysis Using Enzymes and Microorganisms*. Cambridge: Cambridge University Press.
- Rossi, C., Fauve, A., Madesclaire, M., Roche, D., Davis, F. A. & Reddy, R. T. (1992). Microbiological and chemical methods in the asymmetric oxidation of sulfides: a comparative study for the preparation of (S)-vinyl sulfoxides. *Tetrahedron Asymmetry* 3, 629–636.
- Satoh, T. & Kurihara, F. N. (1987). Purification and properties of dimethylsulfoxide reductase containing a molybdenum cofactor from a photodenitrifier, *Rhodobacter sphaeroides* f.s. *denitrificans*. *J Biochem* 102, 191–197.
- Schindelin, H., Kisker, C., Hilton, J. C., Rajagopalan, K. V. & Rees, D. C. (1996). Crystal structure of DMSO reductase: redox-linked changes in molybdopterin coordination. *Science* 272, 1615–1621.
- Schneider, F., Lowe, J., Huber, J., Schindelin, H., Kisker, C. & Knaeblein, J. (1996). Crystal structure of dimethylsulfoxide reductase from *Rhodobacter capsulatus* at 1.88 Å resolution. *J Mol Biol* 263, 53–69.
- Shaw, A. L., Hanson, G. R. & McEwan, A. G. (1996). Cloning and sequence analysis of the dimethylsulfoxide reductase structural gene from *Rhodobacter capsulatus*. *Biochim Biophys Acta* 1276, 176–180.
- Simala-Grant, J. L. & Weiner, J. H. (1996). Kinetic analysis and substrate specificity of *Escherichia coli* dimethylsulfoxide reductase. *Microbiology* 142, 3231–3239.
- Solladie, G. (1981). Asymmetric synthesis using nucleophilic reagents containing a chiral sulfoxide group. *Synthesis* 3, 185–196.
- Styrvold, O. B. & Strom, A. R. (1984). Dimethylsulphoxide and trimethylamine-N-oxide respiration of *Proteus vulgaris*. *Arch Microbiol* 140, 74–78.
- Weaver, P. F., Wall, J. D. & Gest, H. (1975). Characterisation of *Rhodospseudomonas capsulata*. *Arch Microbiol* 105, 207–216.
- Weiner, J., MacIsaac, D. P., Bishop, R. E. & Bilous, P. T. (1988). Purification and properties of dimethylsulfoxide reductase, an iron-sulfur molybdoenzyme of *Escherichia coli*. *J Bacteriol* 170, 1505–1510.
- Weiner, J., Rothery, R. A., Sambasivarao, D. & Trieber, C. A. (1992). Molecular analysis of dimethylsulfoxide reductase: a complex iron-sulfur molybdoenzyme of *Escherichia coli*. *Biochim Biophys Acta* 1102, 1–18.

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