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Preparative asymmetric deoxygenation of alkyl aryl sulfoxides by *Rhodobacter sphaeroides* f.sp. *denitrificans*

Mitsuru Abo,* Akira Okubo and Sunao Yamazaki

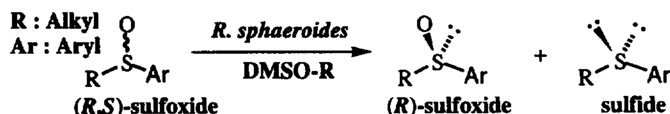
Department of Applied Biological Chemistry, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113, Japan

Abstract: Preparative enantioselective deoxygenation of racemic alkyl aryl sulfoxides by intact cells of *Rhodobacter sphaeroides* f.sp. *denitrificans* was examined. In methyl *p*-substituted phenyl sulfoxides, (*S*)-enantiomers were exclusively deoxygenated while enantiomerically pure (*R*)-isomers were recovered in good yield. For poor substrates such as ethyl phenyl sulfoxide, the repetition of the incubation after removing the toxic product was effective in enhancing the *e.e.* of recovered (*R*)-enantiomers. © 1997 Elsevier Science Ltd. All rights reserved.

Introduction

Enantiomerically enriched sulfoxides have drawn much attention in organic synthesis and have been applied to stereoselective syntheses of natural products.[1] As useful starting materials or synthetic intermediates, alkyl aryl sulfoxides have been prepared by various methods such as Andersen synthesis,[2a] modified Sharpless type oxidation,[2b] biological oxidation[2c] of the corresponding sulfides, and kinetic resolution[2d] of enantiomerically enriched sulfoxides by enantioselective oxidation to sulfones. Among them, the so-called 'biocatalytic' reaction is attractive as an inexpensive and large scale preparative method. In spite of many studies on oxidative reactions[3–6] by means of fungi and bacteria, those on reductive reactions[7,8] to enantiomerically enriched chiral sulfoxides have hardly been reported. In this study we focus on the enantioselective deoxygenation of chiral sulfoxides by a photodenitrifying bacterium as a biocatalyst.

A green mutant of *Rhodobacter sphaeroides* f.sp. *denitrificans* IL106[9] has a variety of respiratory chains and it can utilize O_2, nitrate and dimethyl sulfoxide (DMSO) as electron acceptors.[10] When the bacterium was cultured anaerobically under visible light in the presence of DMSO, DMSO reductase (DMSO-R) was induced in a relatively large amount in its periplasmic space. Previously, we have found that the enzyme has broad substrate specificity and high enantioselectivity.[11] In the case of alkyl aryl sulfoxides, (*S*)-enantiomers are highly selectively deoxygenated to the corresponding sulfides as shown in the scheme.



In general, the enzymatic redox-reaction requires an artificial electron carrier and purified enzyme, which limit the reaction scale. Here, we report an intact cell mediated enantioselective deoxygenation to prepare chiral sulfoxides with high *e.e.* on a preparative scale.

Results and discussion

R. sphaeroides was cultured anaerobically under visible light in the medium containing 0.3% malate as a carbon source and 0.2% DMSO as the terminal electron acceptor.[10] First, we tried to replace

* Corresponding author. Email: abo@hongo.ecc.u-tokyo.ac.jp

Table 1. *E.e.* of recovered sulfoxides by microbial reaction*

substrate	R	Ar	incubation time (h)	recovery (%)	*e.e.* (%) by HPLC
1	Me	C6H5	30	45	>99
2	Me	*p*-CH3-C6H4	120	48	98
3	Me	*p*-Br-C6H4	18	43	100
4	Me	*p*-CH3O-C6H4	36	45	>99
5	Me	PhCH2	54	51	73
6	Et	Ph	120	59	57
7	*n*-Pr	Ph	120	87	3

* *R.sp.* in the end of exponential phase (24 h) was used.

Table 2. Effect of culture conditions on *e.e.* of recovered sulfoxides

entry	substrate	incubation time (h)*	recovery (%)	*e.e.* (%) by HPLC	bacterial growth
1	**1**	24	46	100	72
2	**2**	30	48	98	72
3	**2**	30x2**	40	100	72
4	**4**	24	47	>99	72
5	**5**	36	41	90	72
6	**6**	120	59	57	24
7	**6**	120	49	92	72
8	**6**	120x2**	41	100	72
9	**7**	120x2**	54	21	72

*incubation time after the addition of substrate ** the whole reaction procedure was repeated

DMSO in the culture medium with a series of alkyl aryl sulfoxides listed in Table 1 as respiration substrates for the bacterium, however, both the growth of bacterium and induction of DMSO-R were inhibited because of fairly strong cell toxicity of the substrate and its product (data not shown). Therefore, the strategy to achieve the deoxygenation reaction was as follows; first the bacterium was cultured in the medium containing 0.2% DMSO for more than 24 h until DMSO-R was induced to its full extent before the substrate being added to the culture medium. (*S*)-Enantiomers of phenyl sulfoxide were exclusively deoxygenated and (*R*)-enantiomers remained. In the case of benzyl methyl sulfoxide, the reaction was stopped when approximately half of the sulfoxide was consumed. The *e.e.*'s of the recovered sulfoxides were determined by chiral HPLC analysis.

The *e.e.* of the recovered sulfoxides are summarized in Table 1. Methyl *p*-substituted phenyl sulfoxides **1–4** were obtained in a highly enantiomerically pure state, while **5–7** were recovered with a low *e.e.* The low *e.e.* of **5** accounts for the low enantioselectivity of DMSO-R against **5**, as has been reported earlier.[11]

Effects of culture conditions on *e.e.* of the recovered sulfoxides were examined. The biotransformation was more effective by using cells in stationary phase (72 h of culture) than by those in the exponential phase (24 h of culture) as shown in Table 2 (entry 6, 7). We realized that more DMSO-R was induced after having reached the stationary phase. Use of the cells in the stationary phase in turn shortened the incubation time (entry 1, 2, 4, 5) compared to Table 1. For **2**, **6** and **7** where enantiomeric excesses were not satisfactory, the deoxygenation reaction was repeated (entry 3, 8, 9). Enhancement of *e.e.* by this repeated reaction could be ascribed to the removal of formed sulfide which was toxic to the bacterium. Substrate **7**, on the contrary, had a low *e.e.*, even by the repeated reaction. This may be mainly due to the substrate specificity of DMSO-R that the increase in size of alkyl substituent decreases the reaction rate of alkyl aryl sulfoxide.[11]

The biotransformation on a preparative scale (more than one gram) would be well performed by repeating the whole reaction procedure. For example, 31 mmol (4.82 g) of racemic **2** were subjected to this transformation to give enantiomerically pure **2** (42% yield) as a colorless solid.

In conclusion, this whole cell mediated transformation proved to be useful as a preparative method

Table 3. Retention time of both enantiomers of alkyl aryl sulfoxides

	Compounds	(*S*)-enantiomer (min)	(*R*)-enantiomer (min)
1	Methyl phenyl sulfoxide	11.0	16.0
2	Methyl *p*-tolyl sulfoxide	9.6	16.4
3	*p*-Bromophenyl methyl sulfoxide	11.7	13.7
4	*p*-Methoxyphenyl methyl sulfoxide	12.3	21.3
5	Benzyl methyl sulfoxide	11.4	13.3
6	Ethyl phenyl sulfoxide	9.9	15.2
7	Phenyl propyl sulfoxide	11.0	16.6

for enantiomerically pure starting materials or synthetic intermediates. From these results we propose that the procedure is applicable as a supplementary method to enrich the enantiomeric excess of alkyl aryl sulfoxides. Synthetic application of this biotransformation is now in progress.

Experimental

HPLC analysis was performed by a Jasco-900 system at room temperature, using a Daicel Chiralcel OB-H column (0.46 cm × 25 cm) and UV detector (254 nm). Capillary electrophoresis (CE) was performed on a Waters Quanta-4000E system. Column chromatography was performed on silica gel (60–230 mesh).

Preparation of substrates

Racemic alkyl aryl sulfoxides were prepared by the $NaIO_4$ oxidation of the corresponding sulfides, which are commercially available or synthesized according to the method of Ipatieff *et al.*[12]

Microorganism and biotransformation: general procedure

R. sphaeroides,[9] which was cultured in screw capped bottles filled with the malate-basal salt medium[10] (250 ml) containing DMSO, was grown until it had reached the end of exponential phase (24 h) or the stationary phase (72 h). To this culture medium, 250 mg (1.14 mmol) of **3** was added and further incubated. The reaction was monitored by CE in the mode of MEKC (micellar electrokinetic chromatography).[13] At various time intervals an aliquot (200 μl each) was taken from culture medium and filtered. It was diluted to 500 μl with SDS borate–phosphate buffer and introduced into the capillary (75 μm i.d. × 60 cm). CE was performed at 15 kV, detected at 214 nm and the sulfoxide was quantified.[14] When approximately half of the substrate in the culture medium was consumed, the reaction was stopped and the cells were removed by centrifugation at 8900×g for 25 min. The supernatant was extracted with chloroform (3×100 ml) and the organic layer was dried over anhydrous sodium sulfate, purified by silica gel chromatography (*n*-hexane:ethyl acetate (2:1)–ethyl acetate). 108 mg of **3** was obtained in a solid state (43% yield). Its *e.e.* was determined by chiral HPLC analysis.

Chiral HPLC analysis

The mobile phase was *n*-hexane:2-propanol (1:1), and the retention time was listed in Table 3.

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