Pergamon

Bioorganic & Medicinal Chemistry, Vol. 3, No. 2, pp. 109–112, 1995

0968-0896/95 $9.50 + .00

0968-0896(95)00004-6

Enantioselective Deoxygenation of Alkyl Aryl Sulfoxides by DMSO Reductase from *Rhodobacter sphaeroides* f.s. *denitrificans*

Mitsuru Abo, Makoto Tachibana, Akira Okubo and Sunao Yamazaki

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

Abstract—The substrate specificity and enantioselectivity of DMSO reductase from *Rhodobacter sphaeroides* f.s. *denitrificans* were studied on a series of alkyl aryl sulfoxides as substrate. The enzyme was found to catalyze deoxygenation of (*S*)-sulfoxides predominantly. (*R*)-Sulfoxides were recovered with a high enantiomeric excess.

Introduction

Chiral sulfoxides have been widely used for asymmetric syntheses in recent years and excellent reviews have been reported.[1] As preparative methods for chiral sulfoxides, chemical methods such as the Andersen synthesis[2] and a modified-Sharpless epoxidation method[3] as well as biological methods including biotransformation and enzymatic reactions[4] have been reported. At present, chemical and biological methods are complementary to obtain both enantiomers with a high *e.e.*

Biological methods for asymmetric oxidation of prochiral sulfides have been developed, but, little has been reported on asymmetric deoxygenation of racemic sulfoxides. Although the optical enrichment of sulfoxides recovered from the cultures of *Mortierella isabellina*, *Aspergillus foetidus* and *Helminthosporium* species have been reported,[5] the *e.e.* of recovered sulfoxides was low and the deoxygenation has not been well investigated, compared with the sulfoxidation.

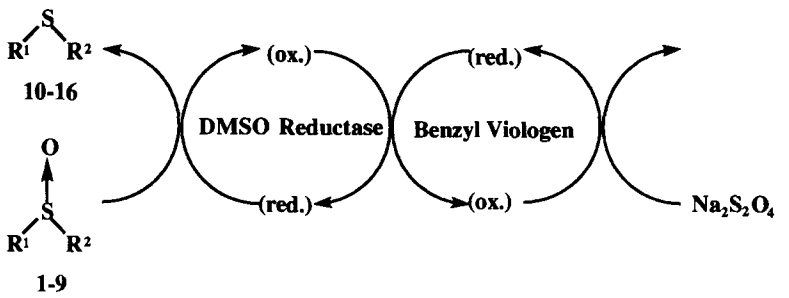
We have been interested in the substrate specificity of DMSO reductase from a photosynthetic bacterium, *Rhodobacter sphaeroides* f.s. *denitrificans* and in the course of our study we found enantioselective deoxygenation of methyl phenyl sulfoxide (MPSO) by the enzyme.[6] Herein, the substrate specificity and enantioselectivity of this enzyme for several alkyl aryl sulfoxides as substrate are reported.

Results And Discussion

DMSO reductase is induced in the periplasmic space of the cell when *R. sphaeroides* f.s. *denitrificans* is cultured anaerobically under light in the presence of DMSO as a terminal electron-acceptor in DMSO respiration.[7] The enzymatic reaction is reconstituted *in vitro* by coupling with an appropriate electron donor. In the present case, benzyl viologen was used, which is reduced by $Na_2S_2O_4$ as shown in the scheme.

The relative reaction rates were measured by the reductase assay system, reported previously[7] and the results are listed in Table 1.

Enantioselectivity of DMSO reductase for the substrates **1–7** was confirmed by a modified assay system,[6] illustrated in the scheme. The conversion of deoxygenation reaction was monitored by the increase in absorbance of the corresponding sulfides **10–16**, using the absorption coefficients summarized in Table 3 (experimental part). The reaction was stopped when about half of the substrate was reduced. The reaction



Scheme.

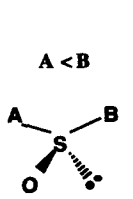
Table 1. Relative reaction rates of alkyl aryl sulfoxides

Compounds	R^1	R^2	Relative Rate*
1	Ph	Me	150
2	*p*-CH_3-C_6H_4	Me	150
3	*p*-Br-C_6H_4	Me	180
4	$PhCH_2$	Me	130
5	Ph	Et	90
6	Ph	*n*-Pr	35
7	Ph	*i*-Pr	35
8	Ph	*t*-Bu	0
9	Ph	Ph	0

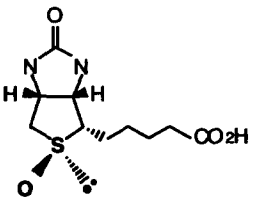
*Rate relative to that of DMSO as 100.

period varied from 40 min to 24 h, depending on the substrate. The reaction mixture was then extracted with chloroform and the extract was purified with silica gel chromatography to give optically active sulfoxides. The *e.e.* of recovered sulfoxides was determined by the ^1H NMR shift reagent method and/or comparison of the values of their specific rotation with those reported. The chemical and optical yields are summarized in Table 2.

Several points are worth noting from the results given in Tables 1 and 2. Water soluble substrates such as DMSO, trimethylamine *N*-oxide (TMAO), methionine sulfoxide and MPSO are known to be reduced and we now report that water insoluble alkyl aryl sulfoxides were catalyzed when a co-solvent (20% methanol) was employed. The sulfoxides with either two aryl or bulky alkyl groups **8** and **9** were not substrates for this enzyme. The rate of deoxygenation was sensitive to the steric size of alkyl chain and electronic factors of the *p*-substituents in the aryl group. The increase in size of alkyl chain decreased the reaction rate (**1** > **5** > **6**, **7**). The substrate with an electron withdrawing substituent showed a high reaction rate (**3** > **1**, **2**). On the contrary, the enantioselectivity was not highly dependent on these factors. This enzyme showed a very high enantioselectivity in the case of α-aryl sulfoxides, whereas poor selectivity was found in the case of benzyl methyl sulfoxide (**4**). Every substrate which was subjected to this enzymatic reaction was deoxygenated with the same absolute configuration, i.e. alkyl aryl (*S*)-sulfoxides were digested and (*R*)-enantiomers were recovered. Thus, the required geometry as substrate is proposed as shown in stereoformula **18** where **A** is smaller than **B**. This rule could be applied to biotin sulfoxide. Biotin *d*-sulfoxide was exclusively reduced by this enzyme[16] and its absolute configuration is shown in **19**.



18



19

In conclusion, this enzyme can be applied to the preparation of chiral sulfoxides in organic syntheses because of its high enantioselectivity.

Experimental

Ultraviolet spectra were recorded with a Shimadzu UV-3101 PC spectrometer. ^1H NMR spectra were recorded at 500 MHz with a Jeol GSX-500 spectrometer, using a mixture of $CDCl_3$ and CCl_4 (1:4) as a solvent. The measurement temperature was 60 °C and Eu(hfc)$_3$, tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato]-europium(III), was used as a shift reagent. The specific rotation was measured with a Jasco DIP-317 polarimeter. Column chromatography was performed on silica gel (60–230 mesh).

Preparation of substrates

Thioanisole (**10**), 4-methylthioanisole (**11**), 4-bromothioanisole (**12**), benzyl methyl sulfide (**13**), ethyl phenyl sulfide (**14**) and diphenyl sulfoxide (**9**), were commercial samples. *n*-Propyl phenyl sulfide (**15**), *i*-propyl phenyl sulfide (**16**) and phenyl *t*-butyl sulfide (**17**) were prepared by the method of Ipatieff *et al.*[13]; **15** and **16** were made by treating thiophenol with the corresponding alkyl bromide and **17** was obtained by the reaction of thiophenol and isobutene in the presence of 75% sulfuric acid. Racemic sulfoxides were prepared from the corresponding sulfides **10**–**17**, using sodium metaperiodate as oxidant.[14]

Table 2. Enantiomeric excess of recovered sulfoxide

Compounds	Recovery(%)	$[\alpha]_D$(c,sol.)	*e.e.*(%) by $[\alpha]_D$	*e.e.*(%) by NMR	Absolute Configuration
1	42	+ 172 (1.05, chloroform)[8]	97	100	*R*
2	35	+ 146 (1.26, acetone)[9]	100	100	*R*
3	38	+ 105 (1.11, acetone)[10]	100	100	*R*
4	52	− 53 (1.11, ethanol)[11]	50	48	*R*
5	40	+ 188 (1.23, acetone)[12]	100	100	*R*
6	45	+ 214 (0.93, acetone)		100	*R*
7	47	+ 200 (1.05, acetone)		100	*R*

Preparation of DMSO reductase

R. sphaeroides f. s. *denitrificans* IL106 was obtained from Professor T. Satoh of Hiroshima University, Japan. This strain is a green mutant of a purple photosynthetic bacterium[17] and it was cultured anaerobically under visible light in the presence of 0.2% DMSO at 30 °C for 24 h. DMSO reductase was purified according to the method reported previously.[7]

Measurement of relative reaction rate

The initial rates were determined by the decrease in absorbance at 600 nm of benzyl viologen, using the reaction mixture reported previously.[7] One hundred per cent activity corresponds to 0.05 U of reductase activity per mL with DMSO as substrate. One unit corresponds to 1 μmol of benzyl viologen oxidized per min.

Enzymatic deoxygenation: typical procedure

The sulfoxide (0.6 mmol) was added to the mixture of the PIPES buffer (50 mM, pH 7, 48 mL) and methanol (16 mL), containing 3 μmol benzyl viologen, and 2.7 units of DMSO reductase. The reaction was started by addition of 12 mL of a sodium dithionite solution (20 mg mL^{-1} of sodium hydrosulfite in 0.2 M sodium hydrogen carbonate). The temperature was kept at 25 °C during the reaction. The reaction was monitored by the UV spectra of the substrate (**1–7**) and the product (**10–16**), using the absorption coefficients listed in Table 3. When the conversion reached around 50%, the reaction was stopped. The reaction mixture was extracted with chloroform and the extract was dried over anhydrous sodium sulfate and concentrated *in vacuo*. The recovered sulfoxide was further purified over silica gel by stepwise elution with *n*-hexane:ethyl acetate (2:1 for **1–4**; 3:1 for **5–7**) and ended with ethyl acetate.

^1H NMR chiral shift reagent method

^1H NMR were measured according to the method of Holland *et al.*[15] NMR spectra of the methyl proton of **1** with and without a shift reagent have been reported previously.[6] The peak separation was improved when the sample was measured at a higher temperature (60 °C). In the presence of 0.4 eq. of $Eu(hfc)_3$, the methyl singlets for the two enantiomers of **1–4** were sufficiently resolved and employed for the determination of *e.e.* However, the *S*-alkyl β proton signals of **6** (methylene) and **7** (dimethyl) and the *S*-alkyl α proton signals of **5** (methyl) were split due to non-equivalence caused by the chiral sulfur atom. These protons were observed as further splitting to doublets in the presence of shift reagent (ex. **6** for β-CH_2: δ 3.40, 3.52, 3.78, 3.85). Moreover, the *S*-alkyl β proton signals of **5** (methyl) and the *S*-alkyl α proton signals of **6** (methylene) and **7** (methine) were also split in the presence of shift reagent (ex. **6** for α-CH_2: δ 4.95, 5.05, 5.35, 5.49). Among them the *S*-alkyl β proton signals of **5** and **6** and the *S*-alkyl α proton signals of **6** and **7** were sufficiently resolved and they were conveniently employed for the determination of *e.e.* According to the NMR results using a shift reagent, the *e.e.* of recovered sulfoxides (**1–7** except **4**), were determined to be 100%.

UV spectral method

The absorbance of sulfoxides **1–7** and sulfides **10–16** was measured in methanol. The wavelengths and absorption coefficients are summarized in Table 3.

Acknowledgements

This study was supported in part by a Grant-in-Aid for Scientific Research on priority areas (No. 05209205) from the Ministry of Education, Science, and Culture of Japan. The authors are indebted to Dr K. Furihata of the Department of Applied Biological Chemistry, the University of Tokyo, for measuring the ^1H NMR spectra.

References

1. (a) Madsclaire, M. *Tetrahedron* **1986**, *42*, 5459; (b) Walker, A. J. *Tetrahedron: Asymmetry* **1992**, *3*, 961.

2. Andersen, K. K. *Tetrahedron Lett.* **1962**, 93.

3. Pitchen, P.; Dunach, E.; Deshmukh, M. N.; Kagan, H. B. *J. Am. Chem. Soc.* **1984**, *106*, 8188.

4. Holland, H. L. *Chem. Rev.* **1988**, 88, 473.

5. Auret, B. J.; Boyd, D. R.; Breen, F.; Greene, R. M. E.; Robinson, P. M. *J. Chem. Soc. Perkin Trans. 1*, **1981**, 930.

6. Abo, M.; Tachibana, M.; Okubo, A.; Yamazaki, S. *Biosci. Biotech. Biochem.* **1994**, *58*, 596.

7. Satoh, T.; Kurihara, F. N. *J. Biochem.* **1987**, *102*, 191.

Table 3. Wavelengths and absorption coefficients

Sulfoxide	λmax	λ(log ε)		Sulfide	λmax	λ(log ε)	
1	236	236(3.53)	252(3.23)	**10**	252	252(3.93)	236(3.62)
2	235	235(3.62)	255(3.07)	**11**	255	255(3.84)	235(3.49)
3	241	241(3.87)	261(3.36)	**12**	261	261(4.06)	241(3.57)
4	247	247(2.28)	260(2.23)	**13**	260	260(2.50)	247(2.75)
5	239	239(3.48)	255(3.13)	**14**	255	255(3.73)	239(3.42)
6	239	239(3.52)	254(3.25)	**15**	254	254(3.80)	239(3.54)
7	243	243(3.53)	256(3.29)	**16**	256	256(3.61)	243(3.37)

8. Felli, V.; Tarossi, D.; Moutanari, F.; Torre, G. *J. Chem. Soc.* (C) **1968**, 1317.

9. Mislow, K.; Green, M. M.; Laur, P.; Melillo, J. T.; Simons, T.; Temary, Jr, A. L. *J. Am. Chem. Soc.* **1965**, *87*, 1958.

10. Cooke, R. S.; Hammond, G. S. *J. Am. Chem. Soc.* **1970**, *92*, 2739.

11. Mislow, K.; Green, M. M.; Raban, M. *J. Am. Chem. Soc.* **1965**, *87*, 2761.

12. Kamiyama, K.; Minato, H.; Kobayashi, M. *Bull. Chem. Soc. Jpn* **1973**, *46*, 3895.

13. Ipatieff, V. N.; Pines, H.; Friedman, B. S. *J. Am. Chem. Soc.* **1938**, *60*, 2731.

14. Johnson, C. R.; Keiser, J. E. *Org. Synth.* **1966**, *46*, 78.

15. Holland, H. L.; Pöpperl, H.; Ninniss, R. W.; Chenchaiah, P. C. *Can. J. Chem.* **1985**, *63*, 1118.

16. Tachibana, M.; Onoe, H.; Okubo, A.; Yamazaki, S. *Biosci. Biotech. Biochem.* **1995**, *59*, 282.

17. Satoh, T.; Hoshino, Y.; Kitamura, H. *Arch. Microbiol.* **1976**, *108*, 265.

(*Received in U.S.A.* 13 *September* 1994; *accepted* 18 *October* 1994)