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Electrochemical enzymatic deoxygenation of chiral sulfoxides utilizing DMSO reductase

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Abstract

Preparation of enantiomerically enriched sulfoxides by an electrochemical enzymatic system utilizing DMSO reductase was studied. This system consists of a glassy carbon electrode as the working electrode, methyl viologen as the mediator and DMSO reductase from *Rhodobacter sphaeroides* f. sp. *denitrificans* as the catalyst. The (*R*)-enantiomers of chiral sulfoxides in the presence of a variety of functional groups were obtained with high *e.e.* (>97%) by this system. © 2000 Elsevier Science Ltd. All rights reserved.

1. Introduction

Enantiomerically enriched sulfoxides have been widely used in asymmetric syntheses of natural products and their applications have been reviewed.[1,2] Although various methods for the preparation of chiral sulfoxides have been reported,[3,4] there have been few which allow various chiral sulfoxides to be prepared with high chemical yield and high enantiomeric excess. Actually, chemical preparations such as the Andersen method,[5] the modified-Sharpless method[6,7] and biological preparations such as asymmetric oxidation[4,8–10] of prochiral sulfides have been used depending on the application.[11–13]

Previously, we have found that DMSO reductase (DMSO-R) from *Rhodobacter sphaeroides* f. sp. *denitrificans* has broad substrate specificity and high enantioselectivity and developed an alternative preparative method for alkyl aryl sulfoxides with high *e.e.* by means of DMSO-R.[14] In addition, the gram-scale preparation of methyl *p*-tolyl sulfoxide (MTSO) by intact cells of *R. sphaeroides* was also described.[15] However, the microbial preparation of chiral sulfoxides in the presence of a variety of functional groups, which are useful for C–C chain elongation and synthetic modification, resulted in low yield because of the undesirable side-reactions.

On the other hand, studies on electrochemical communication between an electrode and a redox enzyme have attracted much attention and several bio-electrochemical systems utilizing redox enzymes

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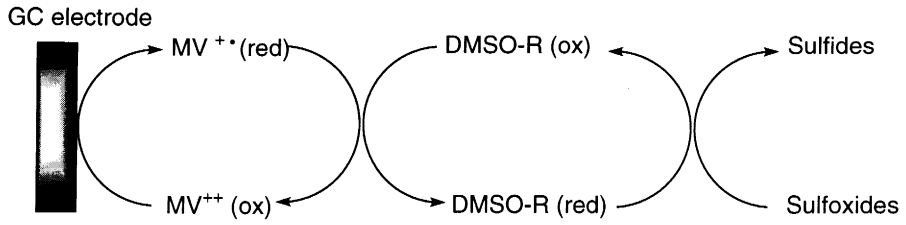
have been reported.[16–19] The electrochemical enzymatic systems could be one of the best preparative methods because the reaction is substrate-specific and it is not necessary to add the chemical reductant. In this respect, we constructed an electrochemical enzymatic system for the preparation of enantiomerically enriched sulfoxides using DMSO-R.

2. Results and discussion

DMSO-R is the terminal electron acceptor in DMSO respiration[20] and catalyzes the deoxygenation of DMSO to dimethyl sulfide. In vitro, DMSO-R can accept electrons directly from artificial electron donors such as viologen dyes in the presence of a chemical reductant (sodium dithionite).[21] In this study, the system involving a glassy carbon (GC) electrode as an electron donor instead of the chemical reductant was constructed as shown in Scheme 1. The radical cation of methyl viologen [MV(red)] carries electron to oxidized DMSO-R and then reduced DMSO-R catalyzes the deoxygenation of sulfoxides to the corresponding sulfides. Because of its fast reaction rate, MV was selected as an electron carrier in this experiment.



Scheme 1.

Methyl viologen, DMSO-R and organic sulfoxides were dissolved in a phosphate buffer. This electrochemical cell consists of a GC electrode as the working electrode, an Ag/AgCl electrode as the reference electrode and a Pt electrode as the counter electrode.

To confirm the electron flow from the GC electrode to the enzyme through the mediator, cyclic voltammograms were recorded. At the applied scan rate of 5 mV/s, these voltammograms showed that the enzyme was able to react with the radical cation MV(red) and catalyze the deoxygenation reaction (Fig. 1A). When the substrate (MTSO) was absent, the catalytic current was not observed (Fig. 1B). In addition, the cyclic voltammogram in the absence of DMSO-R showed that MV(red) did not react directly with the substrate (Fig. 1C).

Utilizing the high enantioselectivity of DMSO-R, enantiomerically enriched sulfoxides were prepared by the kinetic resolution of racemic sulfoxides as shown in Scheme 2. The amount of the substrate remaining in the solution was measured at intervals by means of capillary electrophoresis[22] and the reaction was stopped when more than half of the substrate was deoxygenated. Sulfoxides were extracted with chloroform from the reaction mixture and purified by silica gel chromatography. The *e.e.*s of the recovered sulfoxides were determined by chiral HPLC. The reaction times, *e.e.*s and recovery yields of the sulfoxides are summarized in Table 1.

As shown in Table 1, sulfoxides with a variety of functional groups were recovered with high *e.e.*s by this electrochemical enzymatic reaction system. These results show that this system reflects the high enantio- and chemoselectivity of DMSO-R. In the case of 2-phenylsulfinylethanol **6**, which is a hydrophilic compound due to the hydroxyl and sulfoxide moieties, the recovery yield was improved by MOM (methoxymethyl ether) protection of the hydroxyl group **4**. Sulfinyl esters (**3** and **5**) were recovered with 99% *e.e.* in moderate yield; however, the recovery was lower in order to obtain sulfoxides with *e.e.*s

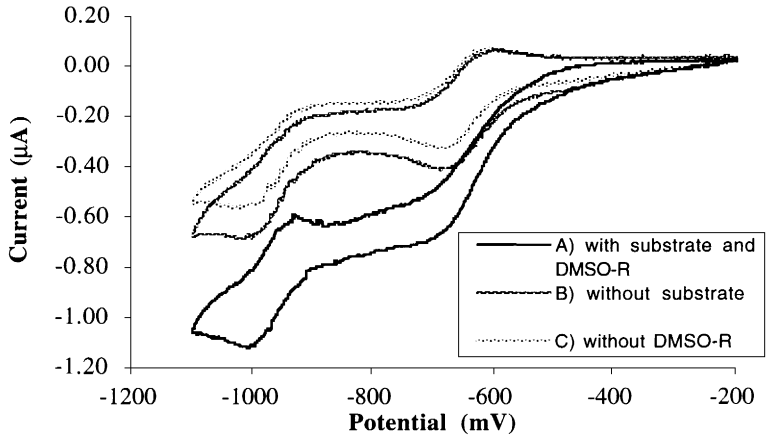
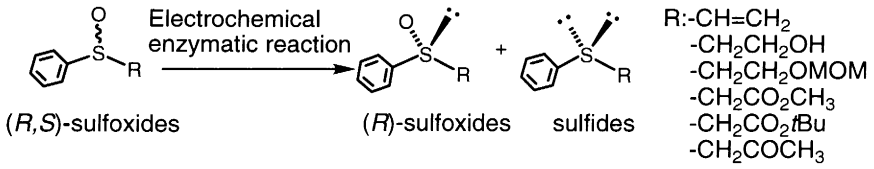


Fig. 1. Cyclic voltammograms of the electrochemical enzymatic system. Cyclic voltammograms were recorded with a GC electrode in 100 mM phosphate buffer (pH 7.4) at the scan rate of 5 mV/s. (A) 10 mM MV, 3.3 mM MTSO as substrate and 3 μM DMSO-R; (B) under the condition (A) without substrate; (C) under the condition (A) without DMSO-R



Scheme 2.

Table 1
Enantiomeric excesses of recovered sulfoxides by the electrochemical enzymatic reaction system

No.	substrate	substrate conc.(mM)	reaction time (h)	enzyme conc.(μM)	recovery yield(%)	*e.e.* (%)
1	Ph-SO-CH=CH2	3.3	3	0.19	29	>99
2	*p* -Tol-SO-Me	3.3	4	0.19	43	>99
3	Ph-SO-CH2-CO2*t* Bu	3.3	4	0.38	38	99
		3.3	5	0.38	31	>99
4	Ph-SO-(CH2)2-OMOM	3.3	6	0.38	44	>99
5	Ph-SO-CH2-CO2CH3	3.3	4	0.38	40	99
		3.3	6	0.38	37	>99
6	Ph-SO-(CH2)2-OH	3.3	5	0.38	31	92
		3.3	8	0.38	26	>99
7	Ph-SO-CH2-CO-CH3	2.2	6	0.38	44	96
		2.2	5	0.76	40	97

>99%. The concentrations of DMSO-R and the substrate in the reaction mixture were varied depending on the reaction rate of the substrate.

One of the characteristics of this method is that the reaction proceeds under mild conditions. If the compounds **3**, **5** and **7** are prepared by means of the corresponding carbanions or sulfinylcarbanions, low temperature and anhydrous conditions are required for the reaction. The mild process of the present electrochemical enzymatic reaction could provide an advantage for the large-scale preparation of enantiomerically pure sulfoxides.

3. Conclusion

We have constructed the electrochemical enzymatic system utilizing DMSO-R and to the best of our knowledge this is the first electrochemical system affording sulfoxides with high *e.e.*s. In asymmetric synthesis this method could, therefore, be useful for the preparation of (*R*)-sulfoxides.

4. Experimental

4.1. Chemicals

Methyl viologen, phenyl vinyl sulfoxide **1**, methyl *p*-tolyl sulfide and thiophenol were commercial samples. Racemic sulfoxides were prepared by the $NaIO_4$ oxidation[23] of the corresponding sulfides. Methyl 2-phenylsulfenylacetate,[24] *t*-butyl 2-phenylsulfenylacetate,[25] 2-phenylsulfenylethanol[26] and phenylsulfenyl-2-propanone[25] were synthesized from thiophenol.

4.2. Preparation of DMSO reductase

DMSO reductase was purified according to the method reported previously.[21]

4.3. Cyclic voltammograms

The cyclic voltammetric measurement was performed using a Model CS-1090 potentiostat (Cypress System Inc.). The current was measured at potentials between −200 and −1100 mV, and the scan rate was 5 mV/s. In the measurement of CVs, a small GC electrode (surface area: ca. 1 mm^2) was used. The reaction cell was 1.5 ml (internal volume) and contained a GC electrode, an Ag/AgCl electrode and a Pt counter electrode. Before the measurement, the cell was capped with a rubber septum and the reaction solution was de-aerated by gentle bubbling with Ar. Then the measurement was started under an Ar atmosphere.

4.4. Capillary electrophoresis (CE) assay

CE was performed on a Waters Quanta-4000E system or Jasco CE-800 system at 25°C. The MEKC method was used for the quantification of sulfoxides.[22]

4.5. Electroenzymatic reaction: general procedure

The GC rod electrode (5 mm ϕ) was used as the working electrode and its surface area in the solution was ca. 250 mm^2. The counter Pt electrode was separated by salt bridge to avoid undesirable side-reactions. The applied voltage was −750 mV versus the Ag/AgCl electrode. The reaction mixture was made up of 10 mM of MV, 0.38 μM of DMSO-R and 3.3 mM of organic sulfoxides dissolved in 18 (or 50) ml of 100 mM phosphate buffer (pH 6.8), containing methanol as co-solvent (10% v/v). Before the reaction was started, the solution was de-aerated by gentle bubbling with Ar and then the cell was sealed by a rubber septum. The reaction was assayed by sampling an aliquot from the reaction mixture and measuring the amount of sulfoxide by CE. When more than half of the substrate was deoxygenated, the reaction was stopped and the mixture was extracted with chloroform. The extract was dried over

anhydrous magnesium sulfate and concentrated in vacuo. The recovered sulfoxide was purified over a silica gel column (hexane:ethyl acetate=5:1–1:1 or chloroform:methanol=9:1) and its *e.e.* was measured by chiral HPLC.

4.6. HPLC analysis

HPLC analysis was performed by a Jasco-900 system at 37°C, using a Daicel Chiralcel OB-H column (0.46×25 cm) and UV detector (254 nm). The mobile phase was hexane:2-propanol=1:1 and the retention times are listed in Table 2. Except for *t*-butyl 2-phenylsulfinylacetate, the absolute configuration of the recovered substrate was determined by comparison of the values of specific rotation (data not shown) with those reported.

Table 2
Retention time of both enantiomers of substrates

No.	Compounds	(*S*)-enantiomer (min)	(*R*)-enantiomer (min)
1	Ph-SO-CH=CH2	10.5	13.6
2	*p*-Tol-SO-Me	8.7	14.7
3	Ph-SO-CH2-CO2*t* Bu*	15.0	16.4
4	Ph-SO-(CH2)2-OMOM	9.4	10.1
5	Ph-SO-CH2-CO2CH3	13.3	14.4
6	Ph-SO-(CH2)2-OH*	19.3	26.0
7	Ph-SO-CH2-CO-CH3	14.2	17.3

*The mobile phase was hexane:2-propanol=9:1

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