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Biotransformation of sulfides by *Rhodocoeccus erythropolis*

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Abstract

A version of *Rhodococcus erythropolis* IGTS8 BKO-53, designed as a model system for the biodesulfurization of crude oil with a high conversion activity of dibenzothiophenes to the corresponding sulfoxides has been used for oxidation of a large number of simple sulfides. A large variety of sulfides were converted to chiral sulfoxides in good yield. Sulfoxide stereoisomers were generally formed as (*R*) configuration in moderate stereochemical purity, but the sulfoxide diasteromers of methionine amino acid derivatives were produced at >90% optical purity.

Keywords: Amino acid oxidation; Biocatalysis; Biotransformation; Enzyme catalysis; *Rhodococcus erythropolis*; Sulfoxide

1. Introduction

The microorganism *Rhodococcus erythropolis* IGTS8 (ATCC 53968) has been studied extensively for its ability to oxidize sulfur-containing compounds, and used, in particular, in the biocatalytic desulfurizaton of fossil fuels [1]. The metabolic pathway for this reaction has focused on dibenzothiophene as a substrate [2], one of the major components of middle-distillate petroleum [3–5]. As a result, most of the research with *R. erythropolis* has been concerned with the mechanism illustrated in Fig. 1, which demonstrated its role as a biocatalyst of dibenzothiophene [6].

The dibenzothiophene desulfurization (*dsz*) operon from *R. erythropolis* IGTS8 encodes three proteins, *DscC*, *DscA*, and *DsaB*, which have been isolated, cloned, mutated, and overexpressed [2,7–10]. The enzymes operating in Fig. 1 show their specific role in sulfur oxidation, with *DscC* of particular interest in converting sulfide to sulfoxide and thence to sulfone: studies on this role have demonstrated that the first step, sufoxidation, is fast and that appreciable amounts of sulfoxide are not further metabolized until the bulk of sulfide is first consumed. Each of the key Dsc enzymes has been purified and characterized [1]; the early step of the pathway which is the *DscC* enzyme is of significance as it can still work for the purpose of some sulfide–sulfoxide–sulfone conversion in spite of other desulfurization reactions when *DscA* and *DscB* are missing.

The use of biocatalysts for the formation of chiral sulfoxides by oxidation of achiral sulfides is not uncommon, but the most frequently used cultures for this role have been fungi [11]. In the bacteria range, only cyclohexanone monoogygenase from *Acinetohacter*

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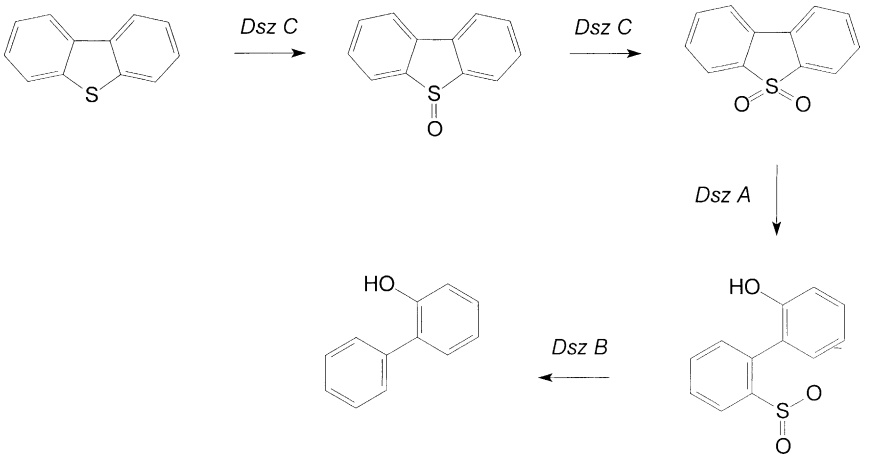

Fig. 1. Biocatalytic desulfurization of dibenzothiophene by *R. erythropolis*.

calcoacetic [12–15] and dioxygenases from *Pseudomonas putida* [16] have been frequently used. In this study, we have examined the use of a series of sulfides for their conversion to chiral sulfoxides, using as a biocatalyst a version of the bacterial species *R. erythropolis* which is missing the enzymes *DscA* and *DscB*, but which has high activity when carrying out biocatalysis reactions by using multiple copies of its *DscC* enzyme [9].

2. Experimental

The preparation of substrates and the analysis of their biotransformation products used the standard methods described previously in [13,17–29], Tables 1–5. The microorganism *R. erythropolis* IGTS8 BKO-53 was originally obtained as frozen cell paste from The Energy BioSystems, The Woodlands, TX [2]. Concentrated frozen paste was resuspended in dibasic potassium phosphate/basic dipotassium phosphate buffer (pH 7, 100 mmol) supplemented with glucose (2% (w/v) final concentration) to form a whole cell preparation of the biocatalyst at a wet weight concentration of 2 g cells per 100 ml of buffer. The substrate was added with 95% ethanol to the cells to give a final concentration of 0.4 mmol sulfide in 1% (v/v) ethanol, and the reaction shaken in a 250 ml orbital shaker at 180 rpm, 30 °C, for 15 h. In a standard 100 ml of buffer as described above, the reaction thus routinely carried a total of 0.4 mmol of sulfide. To check the amount of larger volume reactions, a number of biotransformations of up to 1 l of buffer containing 4 mmol of substrate in a 4 l flask were carried out with no appreciable change in yield.

The cells were then centrifuged (3000 rpm, 15 min), the bacterial precipitate removed and autoclaved, and the supernatant transferred for continuous extraction by dichloromethane. After 2–3 days of continuous extraction the solvent was removed by evaporation, followed by examined by tlc, using ether or 5% methanol–ether as solvent, and then submitted to flash chromatography using a hexane–ethyl acetate

Table 1
Biotransformation of substituted methyl phenyl sulfides

	Substrate	Yield (%)	Configuration	e.e. (%)	Reference
1	$PhSCH_3$	67	(+/−)	2	[17]
2	*p*-CH_3PhSCH_3	72	*R*	62	[17]
3	*m*-CH_3PhSCH_3	60	*R*	10	[18]
4	*o*-CH_3PhSCH_3	95	(+/−)	4	[18]
5	*p*-$CH_3OPhSCH_3$	68	*R*	22	[17]
6	*p*-$FPhSCH_3$	52	*R*	63	[17]
7	*p*-$ClPhSCH_3$	70	*R*	72	[17]
8	*p*-$BrPhSCH_3$	65	*R*	76	[17]
9	*p*-NO_2PhSCH_3	83	*R*	>98	[17]
10	*p*-$CNPhSCH_3$	43	*R*	85	[17]
11	1-$NaphthylSCH_3$	88	*R*	25	[18]
12	2-$NaphthylSCH_3$	79	*R*	62	[18]

Table 2
Biotransformation of substituted benzyl sulfides

	Substrate	Yield (%)	Configuration	e.e. (%)	Reference
13	$PhCH_2SCH_3$	73	*R*	26	[19]
14	$PhCH_2SC_3H_7$	58	*R*	66	[21]
15	$PhCH_2SnC_6H_{13}$	48	*R*	35	[21]
16	$PhCH_2ScycloC_6H_{15}$	85	*R*	24	[21]
17	*p*-$CH_3PhCH_2SCH_3$	70	(+/−)	2	[19]
18	*p*-$C_2H_5PhCN_2SCH_3$	72	*R*	53	[19]
19	*p*-*i*-$C_3H_7PhCH_2SCH_3$	60	*R*	49	[19]
20	*p*-*t*-$C_4H_9PhCH_2SCH_3$	58	(+/−)	3	[19]
21	*p*-$CH_3OPhCH_2SCH_3$	51	*R*	27	[20]
22	*m*-$CH_3OPhCH_2SCH_3$	54	(+/−)	4	[22]
23	*o*-$CU_3OPhCH_2SCH_3$	82	*R*	44	[22]
24	*p*-$CH_3OCH_2PhCH_2SCH_3$	91	*R*	61	[20]
25	*p*-$CH_3COPhCH_2SCH_3$	43	*R*	22	[20]
26	*p*-$CH_3OCOPhCH_2SCH_3$	43	*R*	42	[20]
27	*p*-$FPhCH_2SCH_3$	61	*R*	62	[20]
28	*p*-$CF_3PhCH_2SCH_3$	84	*R*	85	[20]
29	*p*-$ClPhCH_2SCH_3$	61	*R*	65	[20]
30	*p*-$BrPhCH_2SCH_3$	60	*R*	76	[20]
31	*p*-$NO_2PhCH_2SCH_3$	58	*R*	76	[20]
32	*m*-$NO_2PhCH_2SCH_3$	15	*R*	52	[22]
33	*o*-$NO_2PhCH_2SCH_3$	41	*R*	70	[22]
34	*p*-$CNPhCH_2SCH_3$	47	*R*	72	[20]
35	*p*-$CH_3CONHPhCH_2SCH_3$	63	*R*	39	[20]
36	$PhC_2H_4SCH_3$	73	*R*	14	[23]
37	$PhC_3H_6SCH_3$	81	(+/−)	3	[23]
38	$PhSCH_2CN$	50	*S*	13	[13]
39	*p*-$CH_3OPhSCH_2CN$	91	*R*	88	[13]
40	*p*-$BrPhSCH_2CN$	92	*R*	94	[13]

or benzene–ether 10% stepwise gradient, followed by an ethyl acetate–methanol or ether–methanol in 5% stepwise gradient if necessary.

The stereochemical analysis of products was performed with ^{1}H NMR combined with chiral shift reagents, using (*R*)-(−)-*N*-(3,5-dinitrobenzoyl)-α-methylbenzylamine (Kagan reagent [30]) or (*S*)-(+)-α-methoxyphenyl acetic acid (MPAA [31]). ^{13}C NMR and MS spectra were also undertaken [11]. Chiral HPLC was employed for analysis using a Chirobiotic T column with methanol:acetonitrile:acetic acid:triethylamine 545:454:6:1 solvent system at a flow rate of 0.5 ml/min.

Table 3
Biotransformation of dibenzyl sulfides

	Substrate	Yield (%)	Configuration	e.e. (%)	Reference
41	$PhCH_2SPh$	65	*R*	>98	[21]
42	$PhCH_2SCH_2Ph$	55	–	–	[24]
43	$PhCH_2SC_2H_4Ph$	50	*R*	78	[21]
44	$PhCH_2SC_3H_6Ph$	54	*R*	58	[21]
45	*p*-CH_3PhCH_2SPh	62	*R*	82	[25]

3. Results and discussion

A common trend in the results summarized in Tables 1–5 is the use of a wide range of substrates for the oxidation of sulfide, without the formation of appreciable amounts of sulfone. With very few exceptions, the yield of sulfoxide was high; these numbers given in the tables refer to isolated and purified products.

The products listed in Table 1 are all of (*R*) configuration, but are of substantial variation in enantiomeric purity. In general, products with substitution

Table 4
Biotransformations of heterocyclics

	Substrate	Yield (%)	Configuration	e.e. (%)	Reference
46	2-PyridylSCH_3	69	*R*	19	[26]
47	2-PyridylCH_2SCH_3	39	*R*	24	[26]
48	4-PyridylCH_2SCH_3	15	*R*	54	[26]
49	2-PyridylSCH_2Ph	70	*R*	43	[26]
50	4-PyridylSCH_2Ph	51	*R*	23	[26]
51	2-PyridylCH_2SPh	62	*S*	45	[26]
52	4-PyridylCH_2SPh	<5			[26]
53	2-ThiopheneSCH_3	90	*R*	17	[26]
54	2-ThiopheneCH_2SCH_3	90	*R*	11	[26]
55	2-ThiopheneCH_2Sph	85	*R*	49	[26]
56	3-ThiopheneCH_2Sph	80	*R*	34	[26]
57	2-ThiopheneSCH_2Ph	62	*S*	70	[26]
58	2-FurfurylCH_2SPh	46	*R*	43	[26]

in the *para* position are higher than *ortho* or *meta* position, and products with higher electronic withdrawing groups (e.g. *p*-NO_2, and *p*-CN) are also higher than those with the lower electronic withdrawing groups, e.g. *p*-CH_3O.

Table 2 shows a trend similar for sulfoxidation to that observed for Table 1, but with additional information on the size of substituent. Again, the highest e.e. values for simple substituted sulfoxides were obtained for more polar *para* substituted materials (*p*-F, *p*-CF_3, *p*-Cl, *p*-Br, *p*-NO_2, *p*-CN, nos. **27**–**31** and **34**). Large alkyl or aryl substituents such as $PhCH_2S(alkyl)C_6H_{13}$ or *p*-*t*-$C_4H_9PhCH_2SCH_3$ were produced in low e.e. value, as were $PhC_2H_4SCH_3$ and $PhC_3H_6SCH_3$. Notably, the products of highest e.e. values came from substituted cyanomethyl substrates (nos. **39** and **40**) with polar *para* substituents in place, but surprisingly with both OCH_3 and Br in a similar role.

Table 3 deals with a small collection of benzyl sulfides and related compounds as substrates. Not surprisingly, the best substrate was obtained from the ideal substrate, benzyl thioaromatic ether (**41**).

The heterocyclic sulfides listed in Table 4 did not lead to the formation of chiral sulfoxides in high enantomeric purity, but the results of the biotransformation in this series gave an indication of the flexibility of the substrate range that this enzyme can handle.

Even more unusual, however, is the ability for the enzyme of *R. erythropolis* to carry out sulfide oxidations of amino acids such as methionine for the production of sulfoxides. These biotransformation products are in the form of an (R_S) stereochemistry in all cases, independent of the L-(natural) or D-(unnatural, products **58** and **63**) amino acid stereochemistry: this similarity of the stereochemistry of amino acid sulfide oxidation to produce the (R_S)

Table 5
Biotransformations of amino acid sulfides

	Substrate	Yield (%)	Configuration	e.e. (%)	Reference
59	*N*-Phthaloyl-L-methionine methyl ester	49	*R*	88	[27]
60	*N*-MOC-L-methionine methyl ester	54	*R*	83	[28]
61	*N*-MOC-D-methionine methyl ester	53	*R*	>95	[28]
62	*N*-MOC-L-methionine propyl ester	56	*R*	90	[28]
63	*N*-MOC-L-methionine pentyl ester	59	*R*	93	[28]
64	*N*-MOC-L-methionine heptylester	35	*R*	>95	[28]
65	*N*-*t*-Boc-D-methionine ethyl ester	69	*R*	>95	[28]
66	*N*-*t*-Boc-D-methionine pentyl ester	17	*R*	>95	[28]
67	*N*-MOC-*S*-methyl-L-cysteine methyl ester	12	*R*	34	[29]
68	*N*-MOC-*S*-methyl-L-cysteine propyl ester	22	*R*	52	[29]

stereochemistry is identical to that observed during the sulfide-oxidation of similar amino acid conversions by chloroperoxidase dealing with substituted methionine [28] and cysteine [29] derivatives.

4. Conclusions

The use of *R. erythropolis* IGTS8 BKO-53 as a catalyst for the oxidation of sulfides to sulfoxides carries a wide range of structures. The chemical purity of sulfoxide products is generally high, and although the optical purity of sulfoxide is not uniformly high, acceptable levels are generally obtained by crystallization of levels over >80% e.e., leaving purified products with levels of >95% e.e.

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