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Enantioselective oxidation of sulfides to sulfoxides catalysed by bacterial cyclohexanone monooxygenases

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This review article briefly introduces the applications of bacterial cyclohexanone monooxygenases to the enantioselective oxidations of organic sulfur compounds to sulfoxides. High enantioselectivities are observed in the sulfoxidation of alkyl aryl sulfides, disulfides, dialkyl sulfides, cyclic and acyclic 1,3-dithioacetals. The oxidation of alkyl aryl sulfides with flavin dependent microorganisms extends the synthetic interest of this class of enzymes.

The use of sulfoxides as chiral synthons in asymmetric synthesis is a very convenient and reliable strategy, in particular for enantioselective carbon–carbon formation.[1] The sulfoxide functional group is involved in different biological activities and optically pure sulfoxides are of great pharmaceutical interest.[2] However, the use of such sulfoxides has been hampered by difficulties encountered in their preparation, especially for the chiral dialkyl and diaryl sulfoxides. The most successful chemical methods for their asymmetric synthesis involve the Sharpless procedure modified by Kagan[3] and Modena[4] and the use of *N*-sulfinyl oxazolidinones in the presence of nucleophiles.[5]

Alternatively, an enzymatic approach can be adopted. Good to excellent enantioselectivities have been achieved for the sulfoxidation catalysed by isolated enzymes such as pig liver FAD-dependent monooxygenase,[6] monooxygenases from *Pseudomonas* sp.,[7] chloroperoxidase from *Caldariomyces fumago*,[8] toluene and naphthalene dioxygenases[9] and a dioxygenase from *Pseudomonas putida*.[10] Biotransformations with whole cells have mainly employed fungi such as *Aspergillus niger*,[11] *Mortierella* isabellina,[12] *Helminthosporium* sp.,[13] the bacterium *Corynebacterium equi*[14] and, very recently, baker's yeast.[15]

Here we review the results obtained in the biosulfoxidation reaction catalysed by cyclohexanone monooxygenases from *Acinetobacter calcoaceticus* (CYMO) NCIMB 9871 and other bacterial flavin monooxygenases. CYMO is a flavoenzyme of about 60 000 Daltons, active as a monomer which contains one firmly but noncovalently bound FAD unit per monomer.[16] It has a wide potential application in the manufacture of fine chemicals and in organic syntheses based on the Baeyer–Villiger reaction.[16] The only reagents consumed are dioxygen, a reductant and the substrate ketone, which are transformed enantioselectively into the corresponding ester and water. According to the proposed mechanism[16] the 4a-peroxyflavin intermediate acts as an electrophile at the carbonyl carbon. Intramolecular elimination of water from the 4a-hydroxyflavin generates FAD for another catalytic cycle (Scheme 1).

Walsh and co-workers described the synthesis of (*S*)-ethyl *p*-tolyl sulfoxide (64% ee) using CYMO.[17] However, their investigation was not extended to the oxidation of other sulfides. Our interest was to study the stereochemistry of oxidation at the sulfur, catalysed by cyclohexanone monooxygenase, using numerous alkyl aryl sulfides.[18]

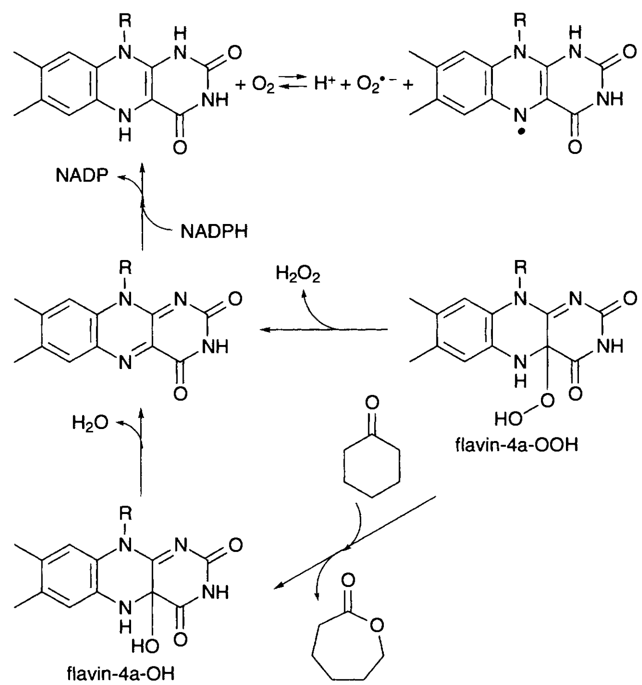
The oxidation of sulfides by the enzyme (reaction 1) was coupled to a second enzymatic reaction to regenerate NADPH; therefore only catalytic quantities of NADPH were required. The regenerating system used was either glucose 6-phosphate and glucose 6-phosphate dehydrogenase (G6PDH) (reaction 2) or L-malate and malic enzyme (reaction 3).

$$\text{R–S–R}' + \text{NADPH} + O_2 + H^+ \xrightarrow{\text{monooxygenase}} \text{R–SO–R}' + \text{NADP}^+ + H_2O \quad (1)$$

$$\text{D-glucose-6P} + \text{NADP}^+ \xrightarrow{\text{G6PDH}} \text{D-gluconate-6P} + \text{NADPH} + H^+ \quad (2)$$

$$\text{L-malate} + \text{NADP}^+ \xrightarrow{\text{malic enzyme}} \text{pyruvate} + \text{NADPH} + CO_2 + H^+ \quad (3)$$

The increase in size of the alkyl chain increased the initial oxidation rates of alkyl aryl sulfides, (Table 1). The benzyl groups were more activating than the phenyl groups but this latter's activation was augmented by the introduction of a substituent in the aromatic ring. With regard to the stereoselectivity of enzymatic reaction, the data (Table 2) indicate that it is highly dependent on substrate structure. Thus, for alkyl aryl sulfides, the optical purity of the products range from 99% ee and (*R*)-configuration with methyl phenyl sulfoxide (entry 1), to 93% ee and (*S*)-configuration with ethyl *p*-fluorophenyl sulfoxide (entry 17). The enzyme showed very high enantioselectivity (99% ee) for *tert*-butyl methyl sulfide (entry 18), but for the two investigated 1,2-disulfides, it was poor (entries 19 and 20).



Scheme 1 Mechanism proposed for oxygen insertion by CYMO in Baeyer–Villiger reaction

The enantiomeric excess of the sulfoxide products did not change appreciably with the progress of the reaction and the oxidation of sulfoxides to the corresponding sulfones was very slow and could not be exploited for kinetic resolution purposes (Scheme 2).

Interestingly, similar results, in terms of enantioselectivity, were obtained using either crude or purified CYMO, so the high sensitivity of cyclohexanone monooxygenase to any structural variation of the substrate is an intrinsic property of a single enzyme. Similar results were obtained with functionalized sulfides[19] and benzyl alkyl sulfides.[20]

The use of macromolecular NADP in a membrane reactor[21] increases the efficiency of coenzyme recycling, a critical step for this kind of biotrasformation. Poly(ethylene glycol)–NADP was used and coenzyme regeneration was carried out with the propan-2-ol–alcohol dehydrogenase system. Both CYMO and alcohol dehydrogenase from *Thermoanaerobium brockii* (ADHTB) maintained high activities with the macromolecular coenzymes (Table 3).

The limiting factor in the number of conversion cycles is the instability of the enzyme, especially in its purified form.

We have proposed an active site model of the enzyme to explain the stereoselectivity of sulfoxidation and to predict the absolute configuration of the products (Fig. 1).[22]

The versatility of cyclohexanone monooxygenase from *Acinetobacter* is further exemplified by its ability to promote enantioselective oxidation of 1,3-dithioacetals.[23] This is a major finding since 1,3-dithioacetals monosulfoxides serve as chiral acyl anion equivalents. In particular, 2-acyl-2-alkyl-1,3-dithiane-1-oxide is an extremely effective moiety for imparting stereocontrol in enolate alkylations and aminations, Mannich reactions, organometallic additions, heterocyclic cycloadditions and so on.[24] *trans*-1,3-Dithiane dioxide can be transformed into thioesters, that act as starting materials in the synthesis of esters, amines, ketones and aldehydes.[25] In spite of their synthetic utility, the preparation of these chirons remains difficult, indeed the oxidation of 1,3-dithiane and of its 2-alkyl derivatives using the Sharpless modified procedure led to monosulfoxides with poor optical purities ($\leqslant$ 30% ee).[26,27] We have found that the CYMO-catalysed oxidation of 1,3-dithiane, 1,3-dithiolane and bis(methylsulfanyl)methane gives enantiomerically pure (*R*)-monosulfoxides with chemical yields ranging from 81 to 94% (Table 4).[23]

Starting from racemic 1,3-dithiane the enzyme was able to oxidize the (*S*)-enantiomer to the corresponding monosulfone faster than the (*R*)-enantiomer, the enantiomeric ratio *E* value

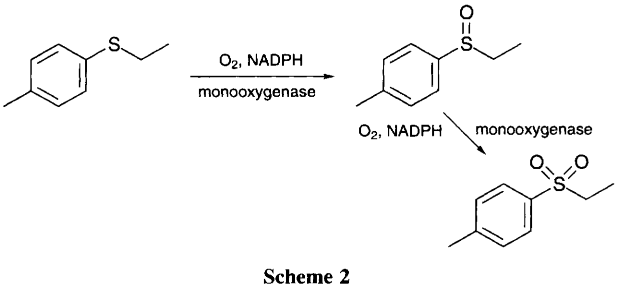
Table 1 Initial oxidation rates by CYMO of some alkyl aryl sulfides to sulfoxides

Sulfides	Relative rate
PhSMe	14
PhSEt	15
PhSPr^i	62
BnSMe	34
p-MeC_6H_4SMe	26
p-MeC_6H_4SEt	34
p-$MeC_6H_4SPr^i$	100
m-MeC_6H_4SMe	22
o-MeC_6H_4SMe	12
p-FC_6H_4SMe	44
p-FC_6H_4SEt	46
p-ClC_6H_4SMe	14
o-ClC_6H_4SMe	5

Table 2 CYMO catalysed oxidation of sulfides to sulfoxides

Entry	Sulfide	Yield (%)	Ee (%)	Sulfoxide configuration
1	PhSMe	88	99	*R*
2	*p*-FC_6H_4SMe	91	92	*R*
3	*p*-MeC_6H_4SMe	90	87	*R*
4	2-pyridyl-SMe	86	87	*R*
5	*p*-$EtOC_6H_4SMe$	92	59	*R*
6	BnSMe	97	54	*R*
7	*o*-$MeOC_6H_4SMe$	81	51	*R*
8	PhSEt	86	47	*R*
9	*m*-MeC_6H_4SMe	90	40	*R*
10	*o*-ClC_6H_4SMe	35	32	*R*
11	PhSPr^i	93	3	*S*
12	*p*-MeC_6H_4SMe	94	37	*S*
13	*p*-ClC_6H_4SMe	78	51	*S*
14	*p*-$MeOC_6H_4SMe$	89	51	*S*
15	*p*-$MeC_6H_4SPr^i$	99	86	*S*
16	*p*-MeC_6H_4SEt	89	89	*S*
17	*p*-FC_6H_4SEt	96	93	*S*
18	Bu^{t}SMe	98	99	*R*
19	BuSSBu	85	32	ND[b]
20	MeSSPr	92	62; 34[a]	ND

[a] For the two regioisomeric thiosulfinates. [b] ND, not determined.



Scheme 2

Table 3 Kinetic constants of CYMO and ADHTB for NADP(H) and PEG–NADP(H)

Enzyme	Coenzyme	V_{max} (rel.)	K_m/µmol dm^{-3}
CYMO	NADPH		<5
	PEG–NADPH[a]	88[c]	33
	PEG–NADPH[b]	62[c]	15
ADHTB	NADP		13
	PEG–NADP	84[c]	28

[a] With cyclohexanone (0.6 mM) as the substrate. [b] With methyl phenyl sulfide (0.6 mM) as the substrate. [c] Relative to the value obtained with native NADP(H) taken as 100.

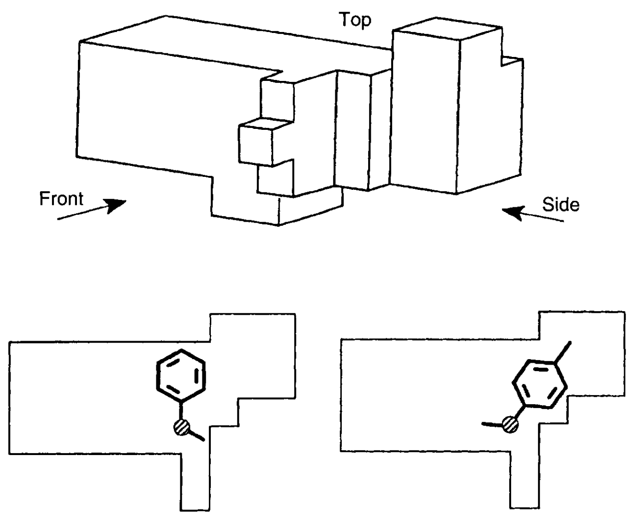
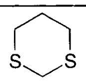
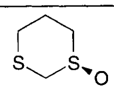
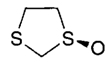
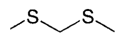
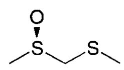


Fig. 1 Active site model of CYMO (upper part). Top perspective view of the active site model showing the preferred binding mode for phenyl methyl sulfide (lower part left) and *p*-Cl-phenyl methyl sulfide (lower part right) (entries **1** and **13** Table 2, respectively).

Table 4 CYMO-catalysed oxidation of 1,3-dithioacetales to monosulfoxides and monosulfones

1,3-Dithioacetal	Monosulfoxide		Yield (%)	Ee (%)	Monosulfone yield (%)
1,3-Dithiane			81	≥98	19
1,3-Dithiolane			94	≥98	6
Bis(methylsulfanyl)methane			92	≥98	8

being 20. As a consequence, enantiomerically pure (*R*)-1,3-dithiane monosulfoxide was obtained as a result of both asymmetric synthesis (v_R/v_S = 24) and kinetic resolution. The same behaviour was determined for bis(methylsulfanyl)-methane, whereas only asymmetric synthesis was operating in the case of 1,3-dithiolane since the v_S/v_R value was as high as 49.

The kinetic parameters for the CYMO-catalysed oxidation of dithioacetals and racemic dithioacetals monosulfoxides are reported in Table 5.

The lower value of K_m and the higher value of k_{cat} for 1,3-dithiane are in agreement with the preference of CYMO towards the dithiane with respect to the monosulfoxide. Interestingly, in the oxidation of thiacyclohexane catalysed by CYMO, the K_m for the sulfoxides was higher (eightfold) than for thiacyclohexane.[16]

The diastereotopic and enantiotopic preference for CYMO-mediated *S*-oxygenation on numerous 2,2-disubstituted and 2-monosubstituted dithioacetals have also been examined along with the effect caused by the replacement of a sulfur atom with an oxygen.[28] The increasing steric bulk present in 2,2-dialkyl-1,3-dithianes and dithiolanes decreased the ee of the obtained monosulfoxides in comparison with the unsubstituted compounds (Table 6). 2,2-Dimethyl-1,3-dithiane yielded preferentially the (*S*)-monosulfoxide, whereas the opposite stereoselectivity was observed with the CYMO-catalysed oxidation of 1,3-dithiane. This result indicates that, not only with the acyclic sulfides, but also with the conformationally more rigid cyclic systems, the steric course of the reaction is highly dependent on substrate structure. With 2-monosubstituted dithioacetals CYMO gave preferentially or exclusively the *trans* monosulfoxide (Table 7).

With 2-methyl-1,3-dithiane the *trans*:*cis* ratio and the ee of the *trans* monosulfoxide increased with reaction time, as was the case for 1,3-dithiane monosulfoxide.[23] 2-Benzoyl-1,3-dithiane was oxidized with high ee to the *trans* monosulfoxide which is a highly selective element of stereocontrol in several reactions.[24] 1,3-Oxathioacetals such as 1,3-oxathiane were transformed into the corresponding monosulfoxides with high chemical and optical yields, showing that the replacement of the sulfur atom with an oxygen does not adversely affect the interaction of the substrate with the enzymes active site. It should be stressed that the asymmetric sulfoxidation method using the modified Sharpless methodology has limited success for unsubstituted 1,3-dithianes and dithiolanes[29] or compounds having simple alkyl groups at C-2,[30] thus making CYMO from *Acinetobacter* the catalyst of choice.

In contrast with several studies on the oxidation of sulfides by purified CYMO, there are very few literature reports using whole cells. Willetts and coworkers have reported the sulfoxidation of some alkyl aryl sulfides, by camphor-grown *Pseudomonas putida* NCIMB 10007, a microorganism containing both NADH and NADPH-dependent Villigerases (enzymes able to catalyse the Baeyer–Villiger reaction).[31] In this case too, the structure of the substrate significantly influenced the enantio-

Table 5 Kinetic parameters for the CYMO-catalysed oxidation of dithioacetals and racemic dithioacetal monosulfoxides

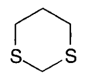
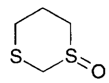
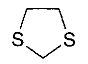
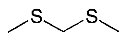
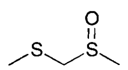
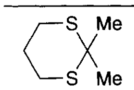
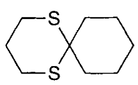
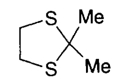
Substrate	K_m/µmol dm^{-3}	k_{cat}/min^{-1}
	33	450
	110	58
	41	309
	76	588
	1300	190

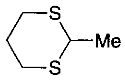
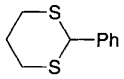
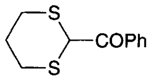
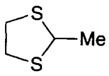
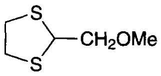
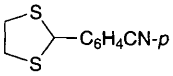
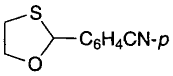
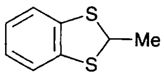
Table 6 CYMO-catalysed oxidation of 2,2-dialkyl dithioacetals to monosulfoxides

Substrate	Conversion (%)	Ee (%)	Absolute configuration
	100	68	*S*
	27	25	—
	100	65	—

selectivity and the stereochemistry of the reaction. The enantiocomplementarity of *Pseudomonas* sp. whole cells to *Acinetobacter calcoaceticus*, present in three of the five substrates reported, was recently shown by Kelly *et al.*[32] *Pseudomonas* sp 9872 was found to oxidize the same sulfides with high and mostly opposite enantioselectivity. The same authors have investigated the biotransformation of methyl phenyl sulfide with two novel organisms *Xanthobacter autotrophicus* DSM 431 and the black yeast NV-2, reported to contain NADH and NADPH dependent Villigerases, respectively. Both species afforded (*R*)-phenyl methyl sulfoxide with 100% ee.

In conclusion, cyclohexanone monooxygenase from *Acinetobacter* shows a wide substrate selectivity towards organic sulfur compounds. Indeed, it is able to oxidize alkyl aryl sulfides, disulfides, dialkyl sulfides, cyclic and acyclic 1,3-dithioacetals and 1,3-oxathioacetals to the corresponding monosulfoxides, the ees being generally high. CYMO exhibits a high diastereopreference for the *trans* isomer with cyclic 1,3-disulfides. Whole cell oxidation of alkyl aryl sulfides with flavin

Table 7 CYMO-catalysed oxidation of monosubstituted cyclic systems to monosulfoxides

			Ee (%)	
Substrate	Conversion (%)	*trans* : *cis*	*trans*	*cis*
	100	10 : 1	95 (1*R*, 2*R*)	not determined
	100	⩾50 : 1	28 (1*R*, 2*R*)	
	90	⩾50 : 1	90	
	100	⩾50 : 1	50 (1*R*, 2*R*)	
	90	⩾50 : 1	56	
	100	15 : 1		100 (1*S*, 2*R*)
	100	34 : 1	10 (1*S*, 2*S*)	100 (1*R*, 2*S*)
	90	4 : 1	50 (1*S*, 2*S*)	8 (1*R*, 2*S*)

dependent microorganisms broadens the synthetic potential of this class of enzymes and favours the potential scale up of these biotransformations. Work is in progress on the preparative scale enantioselective oxidation of 1,3-dithiane[33] and related cyclic and acyclic dithioacetales to the corresponding monosulfoxides using whole cell culture of bacterial monooxygenases.

Stefano Colonna was born in Bologna, Italy, in 1941. He obtained the degree cum laude in Industrial Chemistry at the Bologna University in 1964. In 1980 he was appointed full Professor of Organic Chemistry in the Faculty of Pharmacy of the University of Milan. He was appointed Visiting Professor at the Instituto Quimico de Sarria, Barcelona, Spain, at the University of Toulouse, at the University of Strasbourg in 1991, and at the University of Paris VI in 1993. He is a member of the Directive committee of the C. Erba Foundation. He has published over 120 research papers, several patents and some books. His research interests include carbanion stereochemistry, synthesis and stereochemistry of organic sulfur derivatives, catalytic asymmetric synthesis, stereoselective reactions with natural and synthetic polypeptides, with enzymes and models of enzymes.

Nicoletta Gaggero was born in Chiavari, Genoa, Italy, in 1958. She received her degree in Pharmacy and a PhD in Chemical Sciences from the University of Milan. After post-doctoral work at the CNRS of Toulouse with Professor Bernard Meunier, she continues her research activities at the Faculty of Pharmacy, University of Milan. Her interests include bio-mimethic oxidations.

Piero Pasta was born in Milan, Italy, in 1949. He received his degree in Pharmacy from the University of Milan. Since 1970, he has been a research scientist at the Istituto di Chimica degli Ormoni CNR. His major research interest is focused on the use of enzymes as selective catalysts in organic oxidations.

Gianluca Ottolina was born in Seregno, Milan, Italy, in 1961. He graduated in Organic Chemistry at the University of Milan under the supervision of Professor B. Danieli. After a fellowship with Professor J. B. Jones at the University of Toronto (1990–1991), he joined the Istituto di Chimica degli Ormoni CNR in Milan as research scientist. He specialized in Applied Biotechnology in 1996 at the University of Milan. His main interests are biocatalysis in organic media, enzyme kinetics and molecular modelling.

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