Oxidation of organic cyclic sulfites to sulfates: a new reaction catalyzed by cyclohexanone monooxygenase

Stefano Colonna,*[a]† Nicoletta Gaggero,[a] Giacomo Carrea[b] and Piero Pasta[b]

[a] *Istituto di Chimica Organica, Facoltà di Farmacia, Università di Milano, Via Venezian 21, I 20133 Milano, Italy*
[b] *Istituto di Chimica degli Ormoni, CNR, Via Mario Bianco 9, I 20131, Milano, Italy*

Cyclohexanone monooxygenase from *Acinetobacter* catalyzes the enantioselective oxidation of organic cyclic sulfites to sulfates.

Cyclohexanone monooxygenase (CHMO) (EC 1.14.13.22) from *Acinetobacter* NCIB 9871 is a flavoenzyme of about 60 000 Daltons, active as a monomer which contains one firmly but non-covalently bound FAD unit per enzyme molecule.[1] It has wide potential for application in the manufacture of fine chemicals and in organic synthesis based on the Baeyer–Villiger reaction.[2] The only reagents consumed are dioxygen, NADPH and the substrate ketone, which are transformed enantioselectively into the corresponding ester and water.

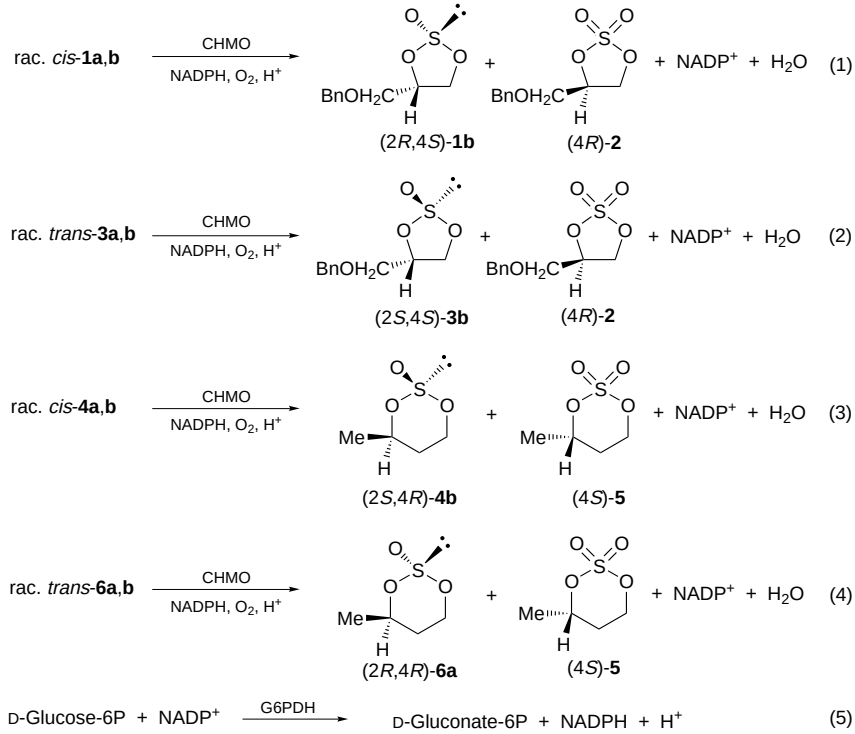
Walsh showed that CHMO can oxygenate heteroatoms, due to the high reactivity of the 4a-hydroperoxyflavin intermediate which acts as an electrophile to trimethyl phosphite and iodide ions, and as a nucleophile to boronic acids.[1] Walsh[3] and our group[4] have also shown that this enzyme catalyzes the asymmetric sulfoxidation of numerous alkyl aryl sulfides with high enantioselectivity. The versatility of CHMO in promoting enantioselective sulfoxidation was recently exploited also with 1,3-dithioacetals[5] and dialkyl sulfides.[6]

We were interested in exploring whether CHMO was able to oxidize organic cyclic sulfites to the corresponding sulfates, a reaction that, to the best of our knowledge, has not been described previously in the literature. Furthermore, this enzymatic procedure could also serve as an alternative route to the only chemical sysnthesis of sulfates of practical interest described so far. This method, developed by Sharpless and Gao, employs sodium metaperiodate as the stoichiometric reoxidant for a ruthenium tetroxide catalyst.[7] Cyclic sulfites and sulfates can be considered synthetic equivalents of epoxides, capable of reacting with a large variety of nucleophiles.[8] In many instances cyclic sulfates are more reactive than oxiranes and, unlike the latter, can lead to disubstitution products.[8]

The diastereoisomeric cyclic sulfites **1**, **3**, **4** and **6**, prepared in racemic form according to the literature,[7] were separated by flash chromatography into their *cis* and *trans* components. The enzymatic oxidation of sulfites (reactions 1–4, Scheme 1) was coupled to a second enzymatic reaction to regenerate NADPH; therefore only a catalytic quantity of NADPH was required. The regenerating system was glucose-6-phosphate and glucose-6-phosphate dehydrogenase (G6PDH) (reaction 5, Scheme 1).[3]

The time-course of reactions (1) and (2) (see Scheme 1) was monitored by HPLC both for conversion and ee. The absolute configurations of the remaining substrates **1b** and **3b** were assigned by comparison of their elution order in chiral HPLC with that of *cis*-(2*R*,4*S*)-**1b** and *trans*-(2*S*,4*R*)-**3b** prepared according to the literature.[9]

The results of the oxidation of 4-benzyloxymethyl-1,3,2-dioxathiolane 2-oxides, *cis*-**1a**, and -**1b** and *trans*-**3a** and -**3b**, reported in Table 1, show that indeed cyclohexanone mono-



Scheme 1

oxygenase in the presence of dioxygen is able to transfer an oxygen atom to the cyclic sulfite in satisfactory chemical yield. The enzymatic reaction is a 'clean' alternative to the chemical oxidation that needs a mixture of CCl_4 and MeCN as solvent. The stereoselective formation of the (4*R*)-sulfate **2** is the result of a kinetic resolution of the starting racemic substrates. The *cis* diastereoisomers (**1a** and **1b**) were more reactive than the *trans* (**3a** and **3b**), as shown by their higher conversion at the same reaction times. The enantioselectivity in the kinetic resolution was also more pronunced for the *cis* sulfite; indeed **1b** was obtained in 94% ee after 60 min, the ee of **3b** being 21%. The stereochemical course of the oxidation is dictated by the absolute configuration of the carbon atom (and not by that of sulfur) since both diastereoisomers afforded the (*R*)-sulfate **2**. The same behaviour was also shown by 4-benzylmethyl- and 4-(benzyl)-1,3,2-dioxathiolane 2-oxide, the *cis* diastereoisomers being more reactive than the *trans*. The kinetic resolution was also more satisfactory for the *cis* sulfites, the ees being in the range 15–70 and 35–50% for the starting material and the reaction products, respectively.

Interestingly, the order of reactivity and the enantioselectivity were reversed in the oxidation of 4-methyl-1,3,2-dioxathiane 2-oxides **4** and **6**. As shown in Table 1, the *trans* sulfites **6a** and **6b** reacted faster than the *cis* sulfites **4a** and **4b** and showed higher stereoselectivity. We do not know whether this behaviour is due to the more flexible nature of the five-membered ring than the six-membered ring,[10] which may affect their interactions with the active site of CHMO or, more generally, to a dramatic influence of the substrate structure on the stereochemical course of the oxidation at sulfur, as we previously found with biosulfoxidation reactions.[4]

Table 1 CHMO catalyzed oxidation of sulfites to sulfates[a]

Substrate	*t*/min	Conversion (%)	Ee (%)	
			Sulfite	Sulfate
1a, **1b**	15	22	26	93
	30	34	44	84
	60	62	94	58
	90	74	⩾99	47
	120	80	⩾99	36
3a, **3b**	15	8	6	66
	30	17	11	56
	60	31	21	47
	90	63	60	35
	120	79	79	25
4a, **4b**	15	7	5	68
	30	17	12	58
	45	27	18	47
	60	32	23	44
	90	60	50	31
	120	69	60	21
6a, **6b**	15	19	20	85
	30	34	32	61
	45	50	54	45
	60	68	71	22
	90	77	80	12
	120	85	93	5

[a] The oxidation of sulfites to sulfates was carried out as follows: the sulfite (10 mM) was reacted at 25 °C, under stirring, in 5 ml of 50 mM Tris–HCl buffer, pH 8.6, containing NADP (1 mM), glucose- 6-phosphate (50 mM), 10 units CHMO [purified as described by Latham and Walsh (ref. 17)] and 100 units of glucose-6-phosphate dehydrogenase. At scheduled times, the reaction mixture was extracted with ethyl acetate (3 × 5 ml), dried and analyzed by chiral HPLC or GLC to determine the degree of conversion and ee. HPLC was carried out in a Chiralpak AS column (Daicel) using *n*-hexane–propan-2-ol (9 : 1) as the mobile phase. The column separated the enantiomers of sulfites *cis*-**1** and *trans*-**3** and of sulfate **2**. The retention times were: **1a**, 9.0; **1b**, 9.9; **3a**, 12.8; **3b**, 17.3; (*R*)-**2**, 30.2; (*S*)-**2**, 31.8 min. GLC was carried out on a CP-cyclodextrin-β-2,3,6-M19 column (Chrompack) under the following conditions: oven temperature 100 to 150 °C; heating rate 1 °C min^{-1}; H_2 as carrier gas. The column separated the enantiomers of sulfites *cis*-**4** and *trans*-**6** and of sulfate **5**. Retention times were: **4a**, 17.8; **4b**, 17.1; **6a**, 10.1; **6b**, 10.6; (*R*)-**5**, 40.2; (*S*)-**5**, 40.8 min.

The absolute configurations of dioxathianes **4** and **6** were assigned by GLC comparison with authentic samples prepared according to the literature.[11]

In conclusion, this new oxidation reaction catalyzed by cyclohexanone monooxygenase, likely *via* 4a-hydroperoxyflavin as intermediate, expands the synthetic importance of this enzyme. The oxygen transfer at sulfur is enantioselective, thus allowing the obtainment of cyclic sulfites and sulfates with high ee under appropriate conditions. We are now investigating the oxidation by CHMO of other sulfites. Since the high electrophilic reactivity of 4a-hydroperoxyflavin is reminescent of that of dioxiranes we are also examining the behaviour of these oxidants in the oxidation of some substrates in organic solvents. It is worth mentioning that, so far, the only reported example of enzymatic oxidation of an organic sulfite is that of dimethyl sulfite, catalyzed by chicken liver sulfite oxidase.[12]

Concerning the scale-up of CHMO catalyzed reactions, we are assessing the possibility of using membrane reactors and macromolecular NADPH, as already done with other coenzyme dependent oxidoreductases.[13,14] However, there are some drawbacks that need to be overcome. One is represented by the stability of the enzyme, which is not very high especially in the presence of bubbling oxygen, which is an essential reagent. Considerable stability improvements have been described recently by feeding oxygen through a thin-walled silicone tube.[15] Another limitation is represented by the low water solubility of substrates. The solution to this problem could be the use of integrated extractive procedures[14] or of hydrophobic resins that act as reservoirs for both substrates and products.[16]

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Notes and References

† E-mail: colonna@imiucca.csiunimi.it

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