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Oxidation of amines catalyzed by cyclohexanone monooxygenase

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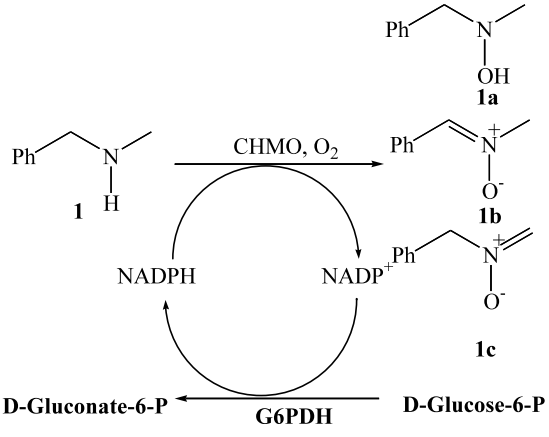
Abstract—Cyclohexanone monooxygenase catalyzed the oxidation of tertiary, secondary and hydroxylamines to *N*-oxides, hydroxylamines and nitrones respectively.

Oxidative biotransformations are amongst the most useful of all identified biologically mediated conversions. They usually involve monooxygenases or diooxygenases that catalyze the insertion of one or two oxygen atoms at a specific point of a molecule, often with high stereo and/or regioselectivity. These very attractive features are now increasingly exploited in the synthesis of many pharmaceuticals and other highly valuable intermediates. In the repertoire of monooxygenases, cyclohexanone monooxygenase from *Acinetobacter calcoaceticus* NCIMB 9871 (CHMO) (EC 1.14.13.22) has been studied most intensively. CHMO is a flavoenzyme of about 60,000 Da, 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 oxidation, which transforms racemic ketones into enantiomerically pure esters. The only reagents consumed are dioxygen, NADPH and the substrate ketone.[2]

Mechanistic studies have shown that the reactive intermediate is 4a-hydroperoxyflavin which can act as an electrophile towards trimethyl phosphite and iodide ions, and as a nucleophile towards boronic acids and ketones.[1] The CHMO can catalyze the asymmetric sulfoxidation of numerous alkyl aryl sulfides,[3] dialkyl sulfides,[4] 1,3-dithioacetals[5] and organic cyclic sulfites.[6] This enzyme also oxygenates heteroatoms such as N, Se and P.[7] Tertiary amines are converted into *N*-oxides, selenides into selenoxides and phosphines into phosphinoxides. Recently, the synthetic potential of this enzyme has been extended by our group to the asymmetric epoxidation of electron-poor olefins.[8]

Another important point is that there are two enzyme systems contained in the mammalian liver, responsible for the oxidation of *N*-substituted amine drugs, in a NADPH and O_2 dependent manner; similar to CHMO, one of them is a flavoprotein.[9]

Finally the nitrones, which are among the expected reaction products of amine oxidation, are useful intermediates for the synthesis of various nitrogen-containing biologically active compounds such as antibiotics, alkaloids, aminosugars and β-lactams.[10] They are excellent spin traps in physiological media and protect the central nervous system against oxidative damage;[11] for instance α-phenyl-*tert*-butyl nitrone exhibits antioxidant and neuroprotective activity.[12]



Scheme 1. CHMO catalyzed oxidation of *N*-methylbenzylamine with in situ coenzyme regeneration.

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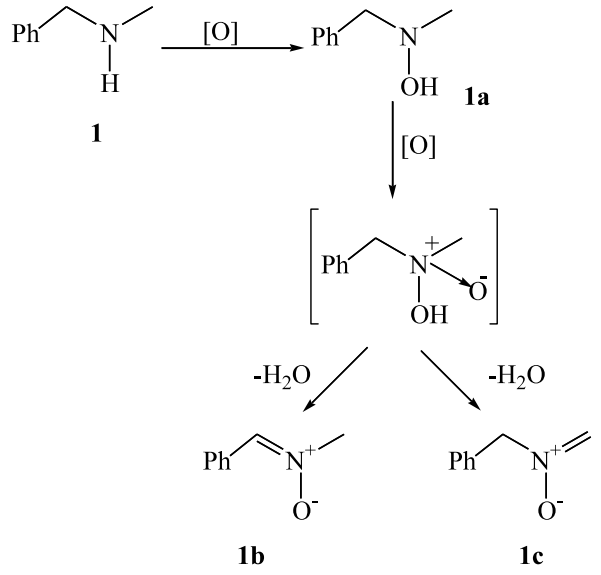
The enzyme used was as a partially purified preparation obtained from an *Escherichia coli* strain in which the gene of CHMO was cloned and overexpressed.[13] The amines (15 mM) were reacted, at 25°C, under stirring, in 0.85 ml of 0.05 M Tris–HCl buffer, pH 8.6, containing $NADP^+$ (0.5 mM), 5 units of CHMO, glucose-6-phosphate (50 mM) and 18 units of glucose-6-phosphate dehydrogenase (G6PDH) that served to regenerate the cofactor (see Scheme 1). After 24 h, the reaction mixtures were extracted with ethyl acetate (3×1 ml) and organic extracts were dried and analyzed. The degree of conversion was determined by HPLC analysis on a Chiralcel OD column using *n*-hexane–propan-2-ol (9:1) as the mobile phase. The products of the reaction were identified by comparison of their eluition order in HPLC with the nitrones and the hydroxylamines prepared according to literature.[10,14] In the case of the tertiary amine the product formed was the amine *N*-oxide.[7]

We have chosen *N*-methylbenzylamine as model substrate in order to understand the mechanism involved in this biooxidation. The enzyme reacts with amine to form first hydroxylamine and then, by further *N*-oxidation, two regioisomeric nitrones in agreement with the study on 4a-hydroperoxyflavin oxidation described by Ball and Bruice.[15] The two nitrones originate by *N*-oxidation of the parent hydroxylamine (see in Scheme 2); their ratio is in favor of the most stable one. The degree of conversion and the V_{max}/K_m values indicate that the catalytic efficiency of CHMO is similar for tertiary and secondary amines (see Table 1). In contrast the oxidation rate of *N*-methylaniline by 4a-hydroperoxyflavin was two orders of magnitude lower than that of *N*,*N*-dimethybenzylamine.[15]

The reaction is quite sensitive to steric effects; indeed methyl-(1-phenyl-ethyl)-amine did not react, thus preventing the possibility of the kinetic resolution of the racemic starting material. Furthermore in the case of *iso*-propyl derivative, replacement of the methyl group with more sterically demanding substituents leads to minor reactivity; the *N*-*tert*-butylbenzyl derivative did not react. Not only steric but also electronic effects influence the reaction rate; indeed *N*-methyl-*p*-chlorobenzylamine and 4-(ethylamino-methyl)-pyridine were recovered unchanged. Dialiphatic secondary amines such as diisopropylamine and pipecoline were not transformed by CHMO.



Scheme 2. Mechanism of oxidation of *N*-methylbenzylamine by CHMO.

The *N*-oxidation of amines by CHMO resembles the sulfur oxidation of organosulfur derivatives, since both represent overall a nucleophilic displacement upon the terminal oxygen of the 4a-hydroperoxyflavin.

Table 1. CHMO catalyzed oxidation of amines

Amines	Products	Conversion (%)	V_{max}/K_m (min^{-1})[a]
N-Methylbenzylamine	$C_6H_5CH{=}N(O)CH_3$	10	87.5
	$C_6H_5CH_2N(O){=}CH_2$	8	
	$C_6H_5CH_2N(OH)CH_3$	80	
N-Methylbenzylhydroxylamine	$C_6H_5CH{=}N(O)CH_3$	36	104.3
	$C_6H_5CH_2N(O){=}CH_2$	16	
N-Ethylbenzylamine	$C_6H_5CH{=}N(O)CH_2CH_3$	30	95.2
	$C_6H_5CH_2N(O){=}CHCH_3$	18	
N-*iso*-Propylbenzylamine	$C_6H_5CH{=}N(O)CH(CH_3)_2$	13	62.5
N-*tert*-Buthylbenzylamine	N.R.		
N-*n*-Buthylbenzylamine	$C_6H_5CH{=}N(O)(CH_2)_3CH_3$	14	58.8
	$C_6H_5CH_2N(O){=}CH(CH_2)_2CH_3$	2	
N-Methyl-*p*-chlorobenzylamine	N.R.		
N-Methylaniline	$C_6H_5N(OH)CH_3$	60	84.6
4-(Ethylaminomethyl)-pyridine	N.R.		
Methyl-(1-phenyl-ethyl)-amine	N.R.		
N,*N*-Dimethylbenzylamine	$C_6H_5N(O)(CH_3)_2$	63	78.4

N.R.: no reaction.

[a] The kinetic constants of CHMO for substrates were determined in 50 mM Tris–HCl buffer at pH 8.6, 25°C, in 1 ml cuvettes, 1 cm path length. The reaction mixture contained CHMO (30 milliunits), 0.1 mM NADPH and 0.4–4 mM substrate. The consumption of NADPH was spectrophotometrically monitored at 340 nm.

In conclusion, we have shown that CHMO reacts with tertiary, secondary and hydroxylamines. In spite of their synthetic limits these biotransformations are very interesting from a mechanistic point of view when compared to the results obtained by 4a-hydroperoxyflavin or by hepatic flavoprotein microsomal oxidase.[16]

Acknowledgements

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