Pergamon

Tetrahedron Letters, Vol. 35, No. 48, pp. 9103-9104, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)01998-3

The Microperoxidase-11 Catalyzed Oxidation of Sulfides is Enantioselective

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

[a]Centro C.N.R. and Istituto di Chimica Organica della Facoltà di Farmacia, Via Venezian 21, 20133 Milano, Italy
[b]Istituto di Chimica degli Ormoni, C.N.R., Via Mario Bianco 9, 20131 Milano, Italy

Abstract: Microperoxidase-11 catalyzed oxidation of alkyl aryl sulfides by H_2O_2 preferentially give the (-)-(S)-sulfoxides (e.e. 16 - 25 %).

Microperoxidase-11 (MP-11) is a heme peptide obtained by the digestion of cytochrome c with proteolytic enzymes.[1,2] It consists of eleven amino acid residues covalently linked via thioether linkages to an iron-protoporphyrin IX having a histidine as axial ligand. Like other peroxidases, MP-11 reacts with H_2O_2 or other peroxides to give two reactive intermediates[3] analogous to compounds I and II of horseradish peroxidase (HRP) that represent states two and one-electron more oxidized than the native ferric form. Such intermediates are able to abstract one electron from the substrate to produce a free radical.[4] In HRP, this species can undergo coupling, disproportionation, or reaction with other substrates or molecular oxygen. However the sulfoxidation of thioanisole suggests, at least in the case of horseradish peroxidase, an atypical two-electron transfer process.[5] Contrary to early reports, recently we[6] and others[5] have found that the oxidation of thioanisole and its analogues by HRP stereoselectively produces the corresponding (S)-sulfoxide with substantial enantiomeric excess (e.e.). Since microperoxidase-11 is a very simple peroxidase that closely mimics horseradish peroxidase, but does not have a substrate binding site, we decided to study its behaviour in the oxidation of organic sulfides with H_2O_2 as oxidant. We report here that asymmetric sulfoxidations by MP-11 are possible under appropriate conditions. We chose methyl p-tolyl sulfide as the model substrate since the stereocentre of the corresponding sulfoxide is optically stable. Note that this sulfide has been used in catalytic asymmetric oxidation catalyzed by iron complexes of chiral "twin coronet" porphyrins.[7] The chemical yield and the e.e. are reported in the Table.[8] Asymmetric induction took place only when H_2O_2 was gradually added to the aqueous buffer. If H_2O_2 was added all at once the sulfoxide was obtained in a racemic form, as a consequence of the complete inactivation of the enzyme due to the excess of the oxidant. It should be pointed out that in all cases the MP-11 catalyzed oxidation of the sulfide was in competition with its spontaneous oxidation by H_2O_2. Therefore, it is likely that in the absence of spontaneous oxidation the enantioselectivity of the process is higher. Racemic methyl p-tolyl sulfoxide was obtained when the reaction was carried out in aqueous-methanol solution as described by Hirobe and coworkers.[9] t-Butyl hydroperoxide was found to be ineffective for MP-11 catalyzed sulfoxidation. It should be emphasized that the oxidation of the sulfide by H_2O_2 with MP-11 is a true enzymatic process which follows Michaelis-Menten kinetics (K_m for sulfide 15 μM; K_m for H_2O_2 459 μM; k_{cat} 80 min^{-1}).

Similar enantioselectivities were observed with the other sulfides tested, namely thioanisole, ethyl phenyl, methyl m-tolyl, methyl p-chlorophenyl sulfide (Table). In all cases the prevailing sulfoxide had the (S) absolute configuration. Interestingly, the stereochemical course is opposite to that we observed with another hemoprotein, chloroperoxidase,[10] but the same as that observed with horseradish peroxidase[5,6] and with soybean hydroperoxide-dependent oxygenase.[11] Therefore, in the sulfoxidation reaction catalyzed by different heme-monooxygenases, the differences in the active site environment control the stereochemistry of the process.

Table. MP-11 Catalyzed Oxidation of Alkyl Aryl Sulfides by H_2O_2.

Sulfides	Yield (%)[a]	e.e. (%)[b]
methyl p-tolyl sulfide	50	24
methyl m-tolyl sulfide	40	25
methyl p-chlorophenyl sulfide	38	24
thioanisole	45	20
ethyl phenyl sulfide	35	16

[a] Based on the sulfide. [b] The absolute configuration of the sulfoxides, as deduced by the elution order on Chiracel OB, was (S).

Spectral and kinetic studies on the oxidation of methyl p-tolyl sulfide by HRP and H_2O_2 have shown that the reaction occurs according to a competition between a two-step "oxygen rebound" mechanism involving compound II and a sulfenium radical cation and another two-step mechanism in which both compound I and compound II react with the sulfide producing a radical cation.[12] Hirobe and coworkers[9] have shown that methyl phenyl sulfide with $H_2{}^{18}O_2$ and MP-11 gives completely ^{18}O labeled methyl phenyl sulfoxide. Our finding that alkyl aryl sulfide oxidation catalyzed by microperoxidase-11 is enantioselective, together with the total ^{18}O incorporation from $H_2{}^{18}O_2$, is consistent with a direct oxygen transfer mechanism, or an oxygen rebound mechanism within the solvent cage. The relatively low value of the enantiomeric excess of the alkyl aryl sulfoxides obtained as reaction products could be a consequence of the low degree of substrate immobilisation by the peptide chain in the active site of this model enzyme. Attempts to improve the enantioselectivity of MP-11 by its modification with short chain peptides are in progress.

REFERENCES AND NOTES

1. Tsou, C.L. *Biochem. J.* **1951**, *49*, 362.
2. Aran, J.; Baldwin, D.A.; Marques, H.M.; Pratt, J.M.; Adams, P.A. *J.Inorg. Biochem.* **1986**, *27*, 227.
3. Cunningam, I.D.; Snare, G.R. *J. Chem. Soc. Perkin Trans.2*, **1992**, 2019.
4. Frew, J.E.; Jones, P. *Adv. Inorg. Biorg. Mech.*, **1984**, *3*, 176.
5. Harris, R.Z.; Newmyer, S.L.; Ortiz de Montellano, P.R. *J. Biol. Chem.*, **9319**, *268*, 1637 and references therein.
6. Colonna, S.; Gaggero, N.; Carrea, G.; Pasta, P. *J. Chem. Soc. Chem. Commun.*, **1993**, 375.
7. Naruta, Y.; Tani, F.; Maruyama, K. *J. Chem. Soc. Chem. Commun.*, **1990**, 1378.
8. The following reaction procedure is representative. The sulfide (36 μmol) and MP-11 (Sigma, Approx 90%) (5.2 μmol) were magnetically stirred in 3.5 ml of 0.1 M phosphate buffer, pH 7.4 at 25°C for 5 min. H_2O_2 (18 μmol) in 540 μl of buffer, pH 7.4, was added in 10 aliquots at 5 min interval. The reaction was quenched with sodium sulfite, extracted with diethyl ether and dried. The product was analysed by HPLC on a Chiracel OB column employing a chiral stationary phase (Daicel) using n-hexane/2-propanol (6:1 v/v) as the mobile phase.
9. Mashino, T.; Nakamura, S.; Hirobe, M. *Tetrahedron Lett.*, **1992**, *33*, 5409.
10. Colonna, S.; Gaggero, N.; Casella, L.; Carrea, G.; Pasta, P. *Tetrahedron Asymmetry*, **1992**, *3*, 95.
11. Blée, F.; Schuber, F. *Biochemistry*, **1989**, *28*, 4962.
12. Perez, H.; Dunford, H.B. *Biochemistry*, **1990**, *29*, 2757.

(*Received in UK* 10 *June* 1994; *revised* 29 *September* 1994; *accepted* 7 *October* 1994)