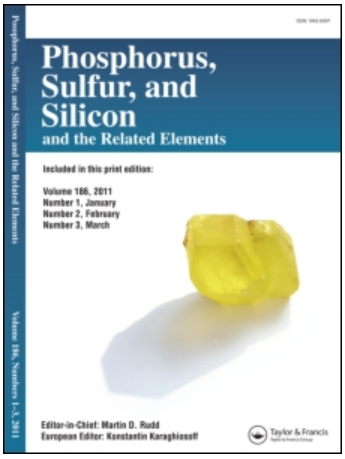


Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

Monooxygenases Catalyzed Enantioselective Silfoxidations

Stefano Colonna[a]; Nicoletta Gaggero[a]
[a] Centro CNR and Istituto di Chimica Organica della Facolta' di Farmacia, Milano, Italy

To cite this Article Colonna, Stefano and Gaggero, Nicoletta(1994) 'Monooxygenases Catalyzed Enantioselective Silfoxidations', Phosphorus, Sulfur, and Silicon and the Related Elements, 95: 1, 103 — 111
To link to this Article: DOI: 10.1080/10426509408034204
URL: http://dx.doi.org/10.1080/10426509408034204

Phosphorus, Sulfur, and Silicon, 1994, Vol. 95–96, pp. 103–111
Reprints available directly from the publisher
Photocopying permitted by license only

MONOOXYGENASES CATALYZED ENANTIOSELECTIVE SULFOXIDATIONS

STEFANO COLONNA AND NICOLETTA GAGGERO
Centro CNR and Istituto di Chimica Organica della Facolta' di Farmacia
Via Venezian 21, 20133 Milano, Italy

A study on the stereochemistry of the oxidation of sulfur by several monooxygenases using numerous organic sulfides as substrates is reported. In most cases the sulfoxidation reaction is highly enantioselective. The various factors that control the enantioselectivity have been examined.

Key Words enantioselectivity, sulfoxides, horseradish peroxidase, chloroperoxidase, microperoxidase - 11, cyclohexanone monooxygenase.

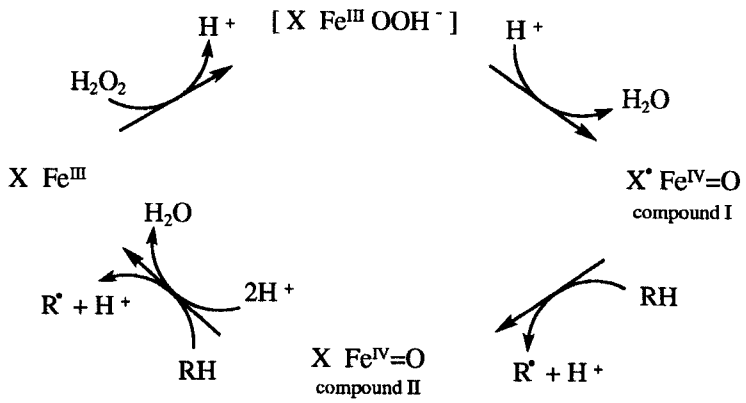
Enantiomerically pure sulfoxides are important chiral synthons for the stereoselective synthesis of natural products and are among the most powerfull stereodirecting groups in the formation of the carbon-carbon bonds. Current interest in chiral sulfoxides also reflects the existence of products with biological properties determined by the configuration of sulfinyl group. Some materials such as liquid crystals are also based on a chiral sulfoxide structure. For these reasons many studies have been devoted at chemical methodologies leading to this structural unit. Good to excellent stereoselectivities have been reported by Kagan[1] and Modena[2] in the oxidation of organic sulfides with t-BuOOH and diethyl tartrate in the presence of titanium i-propoxide. More recently sulfoxides having high enantiomeric excess (e.e.) have been obtained using N-sulfinyloxazolidinones and nucleophiles[3] or N-sulfonyloxaziridines[4].

On the other hand the enzymatic oxidation of organic sulfides to sulfoxides is an important process in the detoxification of xenobiotics, however little is known about the enzymatic mechanism of the process. It has been shown that with flavin containing monooxygenases the sulfoxides are further metabolized to an achiral sulfone[5]. In general organic sulfides are oxidized both in vitro and in vivo by monooxygenases.

Monooxygenases is the general name of a class of enzymes, which incorporate molecular oxygen into an unactivated organic substrate with high stereo and regioselectivity.

Usually dioxygen is cleaved reductively, one oxygen atom being introduced into the substrate, while the other is reduced by two electrons to form water. Monooxygenases utilize various cofactors including flavins, pterins, copper, iron and iron-containing heme. These biotransformations are of great current interest since reveal some structural or mechanistic details of the enzymes involved in the oxidative processes. The heme monooxygenases are subdivided into two types, those depending upon cytochrome P-450 or upon a flavin molecule for oxygen activation and transfer. In this paper we will confine ourselves to chloroperoxidase (CPO), horseradish peroxidase (HRP), as heme enzymes, and to microperoxidase-11 (MP-11) as heme model enzyme. As a flavin dependent monooxygenase we have used cyclohexanone monooxygenase.

Peroxidases catalyze the oxidation of a wide variety of organic compounds, using hydrogen peroxide or other peroxides as oxidants. The peroxidase characteristic activity is one-electron oxidation of the substrates. In the catalytic cycle hydrogen peroxide binds to the ferric ion and its fast reduction to water is coupled to two-electron oxidation of the enzyme. This generates the compound I intermediate, which is reduced in two one-electron steps by substrate molecules, with compound II as the second intermediate (Scheme 1)[6].



Scheme 1.

The substrate radicals produced in the reaction generally evolve nonenzymatically to nonradical products by pathways which are characteristic of each substrate (coupling, dismutation, etc.). Of the two electrons required for peroxide reduction, one comes from Fe(III), while the other, in HRP, comes from the porphyrin, producing a porphyrin radical cation . One electron reduction of compound I gives compound II, in which the Fe^{IV}= O species remains intact but the porphyrin has been reduced. Generally, the reaction of compound II with the substrate is at least 10-20 times slower than that of compound I, and under most steady-state conditions is rate limiting.

An important feature determining the reactivity of peroxidases is the binding of the substrate in the active site. In contrast with P-450 enzymes, which appear to function via the same compound I intermediate, peroxidases are generally unable to transfer the ferryl oxygen atom to the substrate. This has led to the view that the substrate cannot approach the reactive oxo-group in the distal pocket in the peroxidases[7]. Furthermore peroxidases are generally considered poor stereoselective catalysts. This depends on the nature of the reactions promoted by these enzymes, i.e. free radical formation, and the types of substrates generally used in the peroxidase-catalyzed oxidations, e.g. phenols and amines. However, the active site pocket of HRP is capable of chiral recognition of some substrates. Indeed we have been the first to show that HRP catalyzes the enantioselective oxidation of alkyl aryl sulfides to sulfoxides by hydrogen peroxide[8]. Substituted methyl phenyl sulfides were chosen as model substrates since the stereocentre of the corresponding sulfoxide is optically stable. In order to verify the generality of this type of asymmetric synthesis, we also investigated the behaviour of benzyl methyl and methyl-2-pyridyl sulfide.

Asymmetric induction took place only with methyl phenyl and methyl para-substituted phenyl sulfides (30-68% e.e.).

No asymmetric synthesis was observed with methyl ortho-substituted phenyl sulfides, benzyl methyl and 2-pyridyl methyl sulfide. In the cases examined the prevailing sulfoxide had the (S) absolute configuration. It should be pointed out that in all cases the HRP catalyzed oxidation of sulfides is in competition with their spontaneous oxidation by H_2O_2 at a rate dependent on the concentration and nature of the substrate. When taking into account the contribution of the non enzymatic process, the stereoselectivity for the HRP catalyzed oxidation alone is higher. In the case of methyl p-tolyl sulfide the time course of the reaction was monitored; the e.e. of the sulfoxide did not significantly change for the entire reaction period. HRP did not appreciably oxidize racemic methyl p-tolyl sulfoxide to the corresponding sulfone. In

conclusion, we have observed for the first time a substantial enantioselectivity in the horseradish catalyzed oxidation of some aryl methyl sulfides with H_2O_2. This, together with ^{18}O-labeling studies[9] and sulfide binding studies[10] to HRP can be considered as an evidence that aryl methyl sulfides bind near the heme active site. The atypical two-electron oxidation of thioanisole and its p-methyl, p-methoxy and p-nitro analogues by HRP has been investigated a year later by Ortiz de Montellano and coworkers[11]. It produces the (S) sulfoxides in 60-70% e.e.. HRP reconstituted with δ-meso-ethylheme has little peroxidase (guaiacol oxidizing) activity, but shows increased sulfoxidation activity. Studies with $H_2{}^{18}O_2$ with this reconstituted HRP derivative, show that the oxygen in the sulfoxide derives, as it does with the native enzyme, primarly from the peroxide. Kinetic data and differential inhibitory effects of δ-meso-ethylheme reconstitution and phenylhydrazine preincubation indicate that thioanisole and iodide, both of which are subjected to a two electron oxidation, are oxidized at sites distinct from each other HRP derivative. A third binding site is involved in the one-electron oxidation of guaiacol. The enentioselectivity in the HRP oxidation of alkyl aryl sulfides can be considerably increased by molecular engineering[12]. Sequence alignements of HRP isozyme C with cytochrome C and lignine peroxidases as structural templates suggest that Phe-41 is present in the active site of HRP. The Phe-41 → Leu mutant of HRP (F41L-HRP) catalyzes the sulfoxidation with higher turnover numbers and higher enantioselectivity with respect to native or recombinant wild-type HRP. Neither enzyme is active with benzyl methyl sulfide. The e.e. of aryl methyl sulfoxides in all cases is greater than 94%. Sulfoxidation rates for both native and F41L HRP correlate with the σ^+ constants of the substituents and the ρ values are very similar. Mutation of Phe-41 to a threonine also increases the rate of thioanisole oxidation but decreases the enantioselectivity. The results clearly identify Phe-41 as a major factor in determining the binding and orientation of the substrate. The increased polarity of the enlarged pocket in the threonine mutant shifts, according to the authors, the binding of the alkyl group to the smaller pocket and disfavours the formation of the S-enantiomer.

Chloroperoxidase (CPO) is a glycoprotein secreted by the mold Caldariomyces fumago and contains heme b as prosthetic group. It is unique among the peroxidases because it contains a cysteine thiolate as the fifth axial ligand of the heme instead of the classical imidazole ligand.

For this reason many of the spectroscopic properties of CPO are similar to those of P-450[6]. CPO is also unusual because of its versatility: it can catalyze reactions typical of peroxidases, catalases and monooxygenases by H_2O_2 or alkyl

hydroperoxides. It is also almost unique in catalizing halogenation reactions (except fluorination) in the presence of halide ions and H_2O_2. The CPO active site resembles those of classical peroxidases since it contains a distal histidine (His-38), two asparagines (Asn-33 and Asn-37) and an arginine close to the heme group. However, the substrates have access to the heme iron and ferryl oxygen. This is shown, inter alia, by the binding of substrates in the heme pocket, and by oxygen incorporation into the product in the epoxidation of styrene[13], the N-oxidation of arylamines[14] and the sulfoxidation of thioanisoles[15]. These reactions are likely to proceed either through a direct oxygen transfer from the compound I species to the substrate or a P-450 like mechanism involving an initial electron transfer from the substrate to compound I, with formation of a substrate radical species which remains tightly coupled to the active site and compound II, followed by a rapid oxygen transfer from the compound II species. CPO is a very efficient stereoselective catalyst for the oxidation of organic sulfides to sulfoxides. The study of a large series of alkyl aryl, dialkyl and heterocyclic sulfides has shown that various factors, e.g. electronic and particularly steric factors, dramatically affect the outcome of the reaction[16-18]. Excellent yields and very high e.e. (97-100%) have been obtained in the sulfoxidation of a number of sulfides structurally related to methyl p-tolyl sulfide. Binding experiments show that these substrates better fit the active site topology of CPO[10].

It is significant that the trend in affinity of the sulfides for CPO parallels the enantioselectivity pattern observed in the CPO catalyzed oxidation of these substrates. The linear correlation between the binding constants of para-substituted thioanisoles to CPO and the Taft σ_I constants, and the negative slope of the resulting plot, suggest that these substrates act as donors in donor acceptor complexes, involving some residues of the active site. The polar residues in the CPO distal region are candidates for acting as acceptor groups. It is interesting to note that in all cases the prevailing sulfoxide has the (R) absolute configuration. This stereochemical course is opposite to that observed in the HRP and soybean hydroperoxide-dependent oxygenase (SHO)[19], thus indicating that specific effects in the active site control the mode of binding of the substrates and dictate the stereochemistry of the reactions. In the CPO mediated sulfoxidation[17] para-substituted alkyl phenyl sulfides are oxidized with much higher enantioselectivity than the ortho-substituted derivatives.

Microperoxidase-11 (MP-11) is an heme peptide obtained by digestion of cytochrome c with proteolitic enzymes[20]. 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 to give two reactive intermediates, analogous to compounds I and II of HRP[21]. Since MP-11 is a very simple peroxidase that closely mimics HRP, but does not have a substrate binding site, we have studied its behaviour in the oxidation of organic sulfides with H_2O_2 as oxidant[22]. We have found that asymmetric sulfoxidations by MP-11 are possible under appropriate conditions. The oxidation of methyl p-tolyl sulfide 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}). The enantiomeric excess was 24%. Similar enantioselectivities were observed with the other sulfides tested namely thioanisole, ethyl phenyl, methyl m-tolyl, methyl p-chlorophenyl sulfide. In all cases the prevailing sulfoxide had the (S) absolute configuration, i.e. the same stereochemical course as that observed with horseradish peroxidase.

Hirobe and coworkers[23] 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 sulfides oxidation catalyzed by MP-11 is enantioselective, together with the total ^{18}O incorporation from $H_2{}^{18}O_2$ is consistent with a direct oxygen transfer, or an oxygen rebound mechanism within the solvent cage, as already postulated in the case of CPO.

The relative low values of the e.e. of the produced alkyl aryl sulfoxides could be a consequence of the low degree of substrate immobilisation by the peptide chain in the active site of this heme enzyme model.

Cyclohexanone monooxygenase from Acinetobacter is a flovoenzyme of about 60000 daltons[24]. The enzyme is active as a monomer and contains one tighly but noncovalently bound FAD per monomer. It catalyzes the Baeyer-Villiger oxidation of ketones. According to the proposed mechanism[25], NADPH binds to and reduces the enzyme bound FAD. Oxidation of the reduced flavin by O_2 generates E-FAD-4a-OOH, which serves as the oxygen donor and acts as nucleophile at the ketone. The tetrahedral adduct then decomposes by typical Baeyer-Villiger migration of one of the carbon-carbon bonds to yield ester.

Walsh and coworkers[26] have described the synthesis of (S) ethyl p-tolyl sulfoxide (64% e.e.) by the use of cyclohexanone monooxygenase. However, the investigation was not extended to other sulfides. We have undertaken a systematic study of the stereochemistry of oxidation at sulfur catalyzed by cyclohexanone monooxygenase, using as the substrate numerous alkyl aryl sulfides, dialkyl sulfides and dialkyl disulfides[27]. We have found that the structure of the sulfide dramatically influences not

only the enantioselectivity but even the enantiopreference of the enzyme, which yields sulfoxide with optical purities ranging from 99% e.e. and (R)-configuration to 93% e.e. and (S)-configuration.

The investigation has been extended to several alkyl aryl sulfides with the alkyl chain functionalized with Cl, CN, vinyl or hydroxy groups[28]. The structure of sulfide also in this case influences enzyme enantioselectivity. The majority of the sulfoxides had the (S) configuration and the optical purity was in some cases very high. With the cyano derivatives it was possible to move from (R) to (S) sulfoxide, both with e.e.>90%, by simply introducing a methyl group in the para position of the aromatic ring. The sulfides containing keto groups preferentially underwent Baeyer-Villiger oxidation.

In conclusion, despite the apparent conservation of the catalytic mechanism, there are important differences in the catalytic potential of the individual peroxidases. Thus, the problem of the effective ferryl accessibility by organic sulfides in HRP is still unsolved.

Furthermore the different absolute configuration of sulfoxides obtained with different peroxidases is a clear evidence that specific effects in the active site control the mode of binding of the substrates and dictate the stereochemistry of the reaction. Interestingly the progressive drop in enantioselectivity observed in the oxidation of ethyl and isopropyl p-tolyl sulfide with CPO and H_2O_2 is accompanied by a parallel increase of the importance of compound II in the reaction[10]. This reflects increasing HRP- like behaviour in the oxidation and is probably related to the inability of the above substrates to fit properly the active site requirements of CPO in the distal heme pocket, in contrast with the results obtained with all sulfides topographically related to methyl p-tolyl sulfide.

REFERENCES

1. P. Pitchen, M. Deshmukh, E. Dunach and H.B. Kagan, J. Am. Chem. Soc., 106, 8188 (1984).
2. F. Di Furia, G. Modena and R. Seraglia, Synthesis, 325 (1984).
3. D.A. Evans, M.M. Faul, L. Colombo, J.J. Bisaha, J. Clardy and D. Cherry, J.Am.Chem.Soc., 114, 5977 (1992).
4. F.A. Davis, R.T. Reddy, W. Han, P.J. Carrol, J. Am.Chem.Soc., 114, 1429 (1992).

5. E. Benoit, T. Buronfosse, P. Moroni, P. Delatour and J.L. Riviere, Biochem.Pharmacol., 46, 2337 (1993) and references therein.
6. J.H. Dawson, Science, 240, 433 (1988).
7. M.A. Ator and P.R. Ortiz de Montellano, J. Biol. Chem., 262, 1542 (1987).
8. S. Colonna, N. Gaggero, G. Carrea and P. Pasta, J. Chem. Soc.Chem.Comm. 357 (1992).
9. S. Kobahyashi, M. Nakano, T. Kimura and A.P. Schaap, Biochemistry, 26, 5019 (1987).
10. L. Casella, M. Gullotti, R. Ghezzi, S. Poli, T. Beringhelli, S. Colonna and G. Carrea, Biochemistry, 31, 9451 (1992).
11. R.Z. Harris, S.L. Newmyz and P.R. Ortiz de Montellano, J. Biol.Chem., 268, 1637 (1993).
12. S. Ozaki, P.R. Ortiz de Montellano, submitted for publication.
13. P.R. Ortiz de Montellano, Y.S. Choe, G. De Pillis and C.E. Catalano, J.Biol. Chem., 262, 11641 (1987).
14. D.R. Doerge and M.D. Corbett, Chem. Res.Toxicol., 4, 556 (1991).
15. S. Kobayashi, M. Nakano, T. Goto, T. Kimura and A.P. Schaap, Biochem. Biophys. Res. Comm., 135, 166 (1986).
16. S. Colonna, N. Gaggero, A. Manfredi, L. Casella, M. Gullotti, G. Carrea and P. Pasta, Biochemistry, 29, 10466 (1990).
17. S. Colonna, N. Gaggero, L. Casella, G. Carrea and P. Pasta, Tetrahedron Asymmetry, 3, 95 (1992).
18. M. Fu, H. Kondo, Y. Ichikawa, G.C. Cook and C.H. Wong, J. Org. Chem., 57, 7265 (1992).
19. E. Blee and F. Schuber, Biochemistry, 28, 4962 (1989).
20. J. Aron, D.A. Baldwin, H.M. Marquez, J.M. Pratt and P.A. Adams, J.Inorg.Biochem., 27, 227 (1986).
21. J.D. Cunningam and G.R. Snare, J.Chem.Soc.Perkin Trans., 11, 2019 (1992).
22. S. Colonna, N. Gaggero, G. Carrea and P. Pasta, submitted for publication.
23. S. Nakamura, T. Mashino and M. Hirobe, Tetrahedron Lett., 33, 5409 (1992).
24. N.A. Donoghue, D.B. Norris and P.W. Trudgill, Eur.J.Biochem., 63, 175 (1976).
25. C.C. Ryerson, D.P.Ballou and C.Walsh, Biochemistry, 21, 2644 (1982).
26. D.R. Light, D.J. Waxman and C. Walsh, Biochemistry, 21, 2490 (1982).

27. G. Carrea, B. Redigolo, S. Riva, S. Colonna, N. Gaggero, E. Battistel and D. Bianchi, Tetrahedron Asymmetry, 3, 1063 (1992).
28. F. Secundo, G. Carrea, S. Dallavalle and G. Franzosi, Tetrahedron Asymmetry, 4, 1891 (1993).