Tetrahedron Letters, Vol.31, No.22, pp 3163-3166, 1990
Printed in Great Britain

0040-4039/90 $3.00 + .00
Pergamon Press plc

SULFIDE OXIDATION, AMINE N-DEMETHYLATION, AND OLEFIN OXIDATION BY HEME-UNDECAPEPTIDE, MICROPEROXIDASE-11, IN THE PRESENCE OF HYDROGEN PEROXIDE

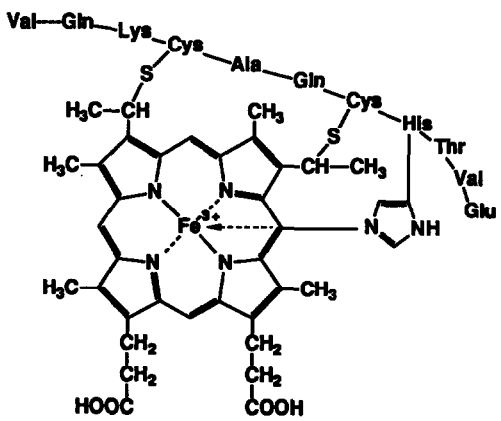
Tadahiko Mashino, Shigeo Nakamura, Masaaki Hirobe

Faculty of Pharmaceutical Sciences, University of Tokyo,

Hongo, Bunkyo-ku, Tokyo 113, Japan

Summary : Heme-undecapeptide, microperoxidase-11, prepared from cytochrome *c* by pepsin digestion, retains the proximal 5th His-18 ligand. Microperoxidase is thought to be a unique peroxidase which does not have a substrate binding site. Compared with hemin-Cl, microperoxidase-11 was an effective catalyst for sulfide oxidation, amine *N*-demethylation, and olefin oxidation in the presence of H_2O_2.

Microperoxidase, MP-11, heme-undecapeptide, is easily prepared by the enzymatic hydrolysis of cytochrome *c* .[1,2] MP-11 retains amino-acid residues 11 - 21, including the proximal His-18 and two thioether bonds between iron-protoporphyrin IX and two Cys residues. It appears to be predominantly monomeric in aqueous-methanol solution, and the proximal imidazole coordinates to the iron tightly at around neutral pH.[3] The peroxidase activity of MPs has been demonstrated in the oxidation of the so-called peroxidase's substrates, phenolic compounds, in the presence of H_2O_2.[4a,b]



Microperoxidase-11 (MP-11)

Most peroxidases are monomeric hemoproteins with the same prosthetic group, iron-protoporphyrin IX, and one proximal His ligand.[5] These enzymes react rapidly with H_2O_2 to form the active species, Compound I, and oxidize the substrate. The active iron-oxo moieties of most peroxidases are buried deeply in the protein, which may prevent an oxo-transfer reaction, and an electronic contact with the substrate often occurs at the heme edge.[6]

MPs are thought to be a unique catalyst since these are very simple hemoproteins, peroxidases, which have an active site including the hemin moiety and the 5th imidazole ligand, but do not have a substrate binding site. As can been seen from a CPK molecular

model of MP-11, all amino acid residues are located on the 5th imidazole site of the heme only and the other site is not occupied.

It is of interest to study the catalytic activity of MPs in comparison with that of native peroxidases and simple organic porphyrin complexes.

MP-11 is available from Sigma Chemical Co., but for our experiment it was prepared from horse heart cytochrome *c* by pepsin digestion and was purified by gel filtration.[2] Our preparation gave a single spot on silica gel TLC. All reactions were carried out in aqueous-methanol solution and the product yields were determined by HPLC or GLC.

The oxidation of methylphenylsulfide and demethylation of *N,N*-dimethylaniline catalyzed by MP-11 or hemin-Cl are shown in Fig. 1-A. MP-11 catalyzed both reactions effectively. The yields of methylphenylsulfoxide and *N*-methylaniline were 100 % based on H_2O_2. Hemin-Cl which was chosen as a simple organic porphyrin complex since it can be dissolved in aqueous-methanol solution proved to be a less effective catalyst compared with MP-11.

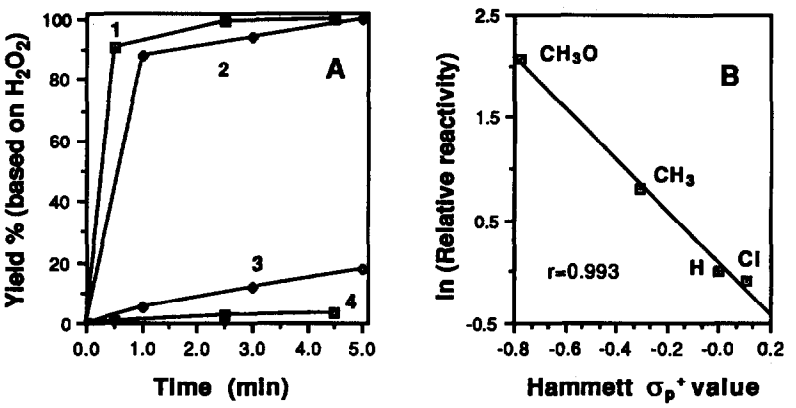
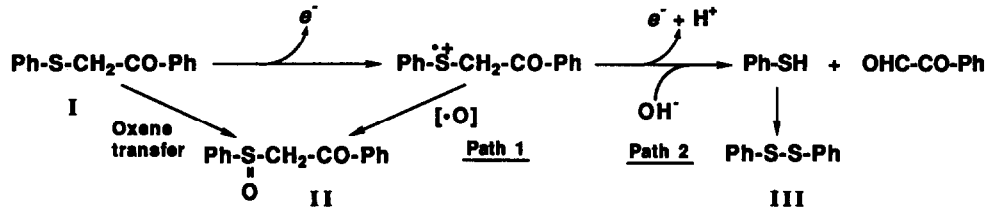


Fig. 1 A. Oxidation of methylphenylsulfide and dealkylation of *N,N*-dimethylaniline: Reaction mixtures contained 1 mM H_2O_2, 5 mM methylphenysulfide (line 1 and 4) or *N,N*-dimethylaniline (line 2 and 3), 50 % methanol, 50 mM sodium phosphate pH 7.4, and 10 μM MP-11 (line 1 and 2), or hemin-Cl (line 3 and 4). Yields of methylphenylsulfoxide and N-methylaniline were based on H_2O_2. **B. Relative reactivities of *p*-substituted methylphenyl sulfides:** Reaction mixtures contained 10 mM each of two kinds of *p*-substituted methylphenylsulfides, 1 mM H_2O_2, 50 % methanol, 50 mM sodium phosphate pH 7.4, and 10 μM MP-11. The relative reactivities were approximated by the ratios of the sulfoxide yields.

Fig. 1-B shows the relative reactivities of *p*-substituted methylphenylsulfides in the MP-11 reaction. They correlated well with the Hammett σ_p^+ values, which provided support for the one-electron oxidation mechanism. Lactoperoxidase (LPO) also catalyzed sulfide oxidation by the same mechanism.[7] In the case of hemin-Cl reaction, the yields of these sulfoxides were too low to accurately measure relative reactivities.

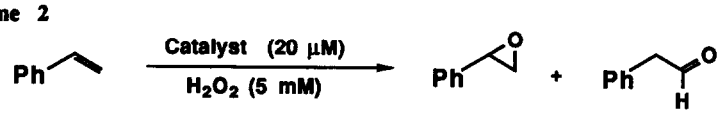
Phenacylphenylsulfide (I) was chosen as a sulfide substrate containing acidic α-protons which are easily lost from the cation-radical intermediate to give the dealkylated product.[8] LPO and H_2O_2 give the S-oxygenated product (II) and diphenyldisulfide (III) which is the stable dealkylated product from I in a ratio of about 1 : 1.[7] The ratio of II to III was about 30 in the MP-11 reaction and about 3 in the hemin-Cl reaction. Our data indicated that the recombination of the S-cation radical and the O-radical bound to the iron-hemin (path 1 in Scheme 1) was much faster than the α-proton loss in the MP-11 reaction (path 2 in Scheme 1), i.e., the imidazole 5th ligand enhances path 1. In the case of LPO, however, the active iron-bound oxomoiety is thought to be buried in the protein, preventing the radical recombination path.

Scheme 1

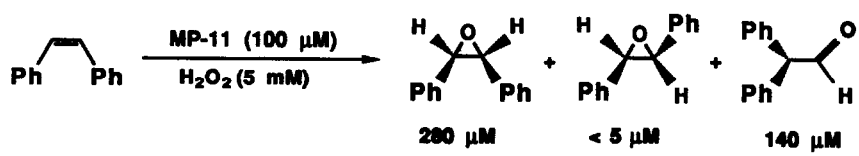


An olefin oxidation by heme compounds is also of interest because olefin was not oxidized by horseradish peroxidase (HRP) and H_2O_2 directly,[9] whereas cytochrome P-450 and chloroperoxidase (CPO) whose 5th ligand is thiolate can catalyze the oxidation.[10] Thiolate ligand seems to be essensial for olefin oxidation, but this assumption may not be conclusive since simple organic porphyrin complexes catalyze olefin epoxidation in the presence and absence of an additive imidazole which loosely coordinates both 5th and 6th ligand sites.[11]

Scheme 2



	SO	PAA
MP-11	80 μM	200 μM
Hemin-Cl	< 5 μM	< 5 μM



MP-11 catalyzed styrene oxidation in the presence of H_2O_2, while hemin-Cl did not under the same conditions (Scheme 2). The main product was phenylacetaldehyde (PAA) in the MP-11 reaction, which was not produced by decomposition of styrene oxide (SO) since SO did not give PAA in the MP-11 reaction. PAA is obtained as a by-product in the reactions of cytochrome P-450[10] or simple organic heme complexes,[12] whereas CPO-catalyzed reaction yields almost the same amount of SO and PAA.[10] *cis*-Stilbene was converted stereospecifically to *cis*-epoxide by MP-11 (the ratio of the *trans*-oxide was less than 1 %) and to the rearrangement product, diphenylacetaldehyde. Another possible rearrangement product, benzylphenylketone, was below detectable amount. The phenyl rearrangement was also dominant in tetraphenylporphinatoiron - PhIO system.[13] This result indicates that the iron-oxo species of MP-11, probably Compound I type porphyrinoxenoid, oxidized *cis*-stilbene directly.[11] These data show that the active species of peroxidase have a potential to catalyze olefin oxidation, but its effectiveness depends on the active site environment.

In conclusion, MP-11 is a unique and simple hemoprotein which is not restricted by consideration of the apoprotein structure. Recently extensive studies have been made to clarify the difference between the reactivity of cytochrome P-450 and that of peroxidases in relation to their structures.[6,9,10,14] MP-11 is thought to be a good peroxidase model for studying the difference. Further mechanistic investigation will be described elsewhere.

References

1. Tsou, C. L., (1951) *Biochem. J.*, **49** 362 - 367.
2. Aron, J., Baldwin, D. A., Marques, H. M., Pratt, J. M., and Adams, P. A., (1986) *J. Inorg. Biochem.*, **27** 227 - 243.
3. Baldwin, D. A., Marques, H. M., and Pratt, J. M., (1986) *ibid*, **27** 245 - 254.
4. a), Adams, P. A., and Adams, C., (1988) *ibid*, **34** 177 - 187. b), Traylor, T. G., and Xu, F., (1990) *J. Am. Chem. Soc.*, **112** 178 - 186.
5. Hewson, W. D., and Hagar, L. P., (1979) The Porphyrins, Vol. 7, pp. 295 - 332. (Dolphin, D., Ed.), Academic Press, New York.
6. Ortiz de Montellano, P. R., (1987) *Acc. Chem. Res.*, **20** 289 - 294.
7. Doerge, D. R., Pitz, G. L., and Root, D. P., (1987) *Biochem. Pharmacol.*, **36** 972 - 974.
8. Watanabe, Y., Numata, T., Iyanagi, T., and Oae, S., (1981) *Bull. Chem. Soc. Jpn.*, **54** 1163 - 1170.
9. Ortiz de Montellano, P. R., and Grab, L. A., (1986) *Mol. Pharmacol.*, **30** 666 - 669.
10. Ortiz de Montellano, P. R., Choe, Y. S., DePillis, G., and Catalano, C. E., (1987) *J. Biol. Chem.*, **262** 11641 - 11646.
11. Labeque, R., and Marnett, L. J., (1989) *J. Am. Chem. Soc.*, **111** 6621 - 6627.
12. Ostovic, D. and Bruice, T. C., (1989) *ibid*, **111** 6511 - 6517.
13. Castellino, A. J., and Bruice, T. C., (1988) *ibid*, **110** 158 - 162.
14. Champion, P. M., (1989) *ibid* **111** 3433 - 3443.

(Received in Japan 7 February 1990)