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Porphyrins in aqueous amphiphilic polymers as peroxidase mimics

Robert A. W. Johnstone,*[a] Anthony J. Simpson[b] and Paul A. Stocks[a]

[a] *Department of Chemistry, University of Liverpool, Liverpool, UK L69 3BX*
[b] *Procter and Gamble Ltd., Newcastle Technical Centre, Newcastle, UK NE12 9TS*

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Water-insoluble porphyrins dissolve in aqueous solutions of amphiphilic polymers to give systems which mimic the action of peroxidase enzymes.

Porphyrins hold an important position in oxidative mechanisms of metabolism, ranging from the oxygen-carrying capacity of haemoglobins to the oxidative reactions of cytochrome and peroxidase enzymes.[1] Mechanisms of reaction, in which an oxygen atom is transferred from a donor (*e.g.* O_2, H_2O_2, hydroperoxides, hypochlorites, iodates, iodosobenzene) to a substrate such as an alkene to form an epoxide or to an alkane or arene to form a hydroxy derivative, have been extensively examined, particularly for cytochrome P450 systems.[2] More recently, there has been considerable development of model cytochrome P450 enzyme systems as catalysts for oxidation.[3] Most research on peroxidases has been done with horse radish peroxidase[4] and sensitive tests have been developed to distinguish peroxidases from cytochromes P450.[5] However, relatively little research into peroxidase mimics has appeared and mostly has not distinguished between peroxidase and cytochrome P450 reaction mechanisms.[6] The present work describes a mimic for a peroxidase, which has intrinsic activity similar to that of horse radish peroxidase.

A variety of water-soluble iron or manganese porphyrins was prepared through sulfonation and metallation of 5,10,15,20-tetraarylporphyrins.[7] Other water-soluble metallo derivatives were prepared from 5,10,15,20-tetrakis(1-oxidopyridin-1-ium-4-yl)porphyrin[8] and from 5,10,15,20-tetrakis{4-[ω-hydroxypoly(ethoxy)ethoxy]phenyl}porphyrin.[8] As a quick test of the capacity of these water-soluble porphyrins for catalysing electron-transfer type oxidation with longevity of action, their ability to bleach a small range of red, orange and blue water-soluble dyes was examined, using H_2O_2 as the primary oxidant.[9] In all cases, 2–30% bleaching of the azo, anthraquinone and phthalocyanine dyes was observed but complete auto-oxidation and destruction of the porphyrin catalyst itself always occurred first and stopped the reaction. Indeed, with H_2O_2 alone and no added dye, the water-soluble manganese or iron 5,10,15,20-tetrakis(4-sulfophenyl)porphyrins were destroyed in 30 s and 2 min, respectively. Because cytochrome and peroxidase enzymes (albeit enveloped in a protein coat) can utilise H_2O_2 and porphyrin in an aqueous environment, it was decided to attempt to mimic the action of these proteins through use of synthetic porphyrins carried in amphiphilic water-soluble polymers. Two such examples of carriers used in the present work are poly(sodium styrene-4-sulfonate-*co*-2-vinylnaphthalene) (PSSS-VN) and poly-(sodium styrene-4-sulfonate-*co*-2-vinylpyridine) (PSSS-VP).[10] It was found that porphyrins in PSSS-VN co-polymers formed systems that behaved like a typical peroxidase (horse radish peroxidase) and unlike P450 cytochromes.

Epoxidation of alkenes with H_2O_2 and P450 metalloporphyrin model catalysts is typically carried out under biphasic conditions.[11,12] Here, manganese(III) 5,10,15,20-tetraphenylporphyrin in aqueous PSSS-VN polymer with added imidazole effected the epoxidation of cyclooctene (48%), 1-methylcyclohexene (29%) and 2,3-dimethylbut-2-ene (7%) in CH_2Cl_2–aq. H_2O_2 but only very slowly and in the poor yields indicated. Significantly, the porphyrin survived these reactions with little auto-oxidation during 48 h. The percentage conversions are not only low but are in the reverse order of reactivity normally found for epoxidation of these alkenes with peroxy acids or cytochrome mimics, and indicate that the epoxidation was not the result of direct oxygen transfer from the metal centre of the porphyrin.

A number of substrates has been used to estimate peroxidase activity.[13] All of those tested in this work (phenols, amines, leuco dyes, ferrocyanide) gave positive responses with the PSS-VN–porphyrin system, and were inactive in the presence of cyanide ion. Most of these substrates do not distinguish cytochrome P450 from peroxidase activity. The most definitive tests for peroxidase activity are generally considered to be colour reactions of guaiacol, mesidine and, particularly, the diammonium salt of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS).[5] The PSSS-VN–porphyrin system showed significant activity in all of the above tests[13] but only the last three[5] were examined in kinetic detail to distinguish peroxidase specifically from cytochrome P450 activity. The graphs in Fig. 1 compare the rates of oxidation of ABTS with hydrogen peroxide alone and also in the presence of authentic horse radish peroxidase or manganese(III) 5,10,15,20-tetraphenylporphyrin in aqueous PSSS-VN polymer. The results have been normalised for the amounts of porphyrin in the

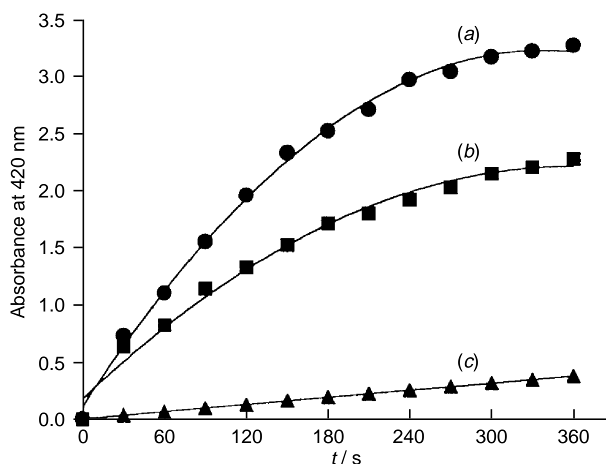

Fig. 1 Graph showing the oxidation of ABTS by H_2O_2 in the presence of (*a*) horse radish peroxidase catalyst, (*b*) Mn^{III} tetraphenylporphyrin–PSSS-VN polymer catalyst, and (*c*) no added catalyst. For (*a*), ABTS stock solution (0.2 ml) was mixed with H_2O_2 stock solution buffered to pH 8 (0.2 ml) and then horse radish peroxidase stock solution (2 ml) was added; the oxidation was monitored over a period of 6 min by the appearance and UV–VIS absorbance of a band at 405 nm due to the ABTS radical cation. After this time, the H_2O_2 had been used up. For (*b*), a similar solution was prepared and monitored but using a porphyrin–PSSS-VN stock solution in place of horse radish peroxidase. For (*c*), only H_2O_2 and ABTS solutions were mixed and monitored at 405 nm. For normalization purposes, the solutions were also monitored at 420 nm (porphyrin Soret band) so as to calculate the molar concentrations of porphyrin in the horse radish peroxidase and in the PSSS-VN system; the ABTS radical cation absorbance was divided by the molar concentration of porphyrin.

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aqueous peroxidase and polymer solutions. Whilst horse radish peroxidase causes faster oxidation of ABTS, the biomimetic porphyrin–polymer system is 70% as fast; in the absence of either the authentic or artificial peroxidase, oxidation of ABTS with H_2O_2 is very slow. Similarly, the comparative effects of horse radish peroxidase and the PSS-VN–porphyrin system as catalysts in the H_2O_2 oxidation of guaiacol and mesidine showed that the authentic peroxidase and its mimic gave comparable rates of reaction and both exhibited a greatly increased rate compared with that for reaction with H_2O_2 alone. When the amphiphilic PSSS-VN polymer was replaced by the more hydrophilic PSSS-VP, the normally water-insoluble porphyrins dissolved as usual but the peroxidase-like activity was greatly reduced. It is notable that, in all these oxidations, the simple water-insoluble porphyrins such as manganese(III) 5,10,15,20-tetrakis(4-methoxyphenyl)porphyrin, when solubilised in water containing the amphiphilic polymer, were destroyed only very slowly as compared with the rapidity of auto-oxidation of similar water-soluble porphyrins in the absence of polymer. This stability suggests that the water-insoluble porphyrin molecules in the inner hydrophobic sphere of the PSSS-VN polymer must be isolated from each other so as to prevent mutual oxidation; the behaviour is characteristic of natural peroxidases.

The substrates to be oxidised (ABTS, guaiacol and mesidine) are soluble in water. For horse radish peroxidase, their approach to the metal centre in the porphyrin is prevented by the protein coat. For the PSSS-VN–porphyrin system it is probable that the porphyrin is not only held in the hydrophobic core of the PSSS-VN polymer in water but is isolated by intercalation between the many naphthalene residues in such a way that the oxidised metal centre is not open to approach by the substrate. Aggregation of polycyclic aromatic species through π–π interactions are well-known through UV effects[14] and it was found here that, in these PSSS-VN polymer–porphyrin systems, the Soret band near 420 nm is significantly broadened and reduced in height as compared with the free porphyrin.[8] Also, Mössbauer spectroscopy of the solid isolated by evaporation of water from a PSSS-VN polymer–iron porphyrin system revealed a small shift in the iron peak, suggesting electronic interaction of the porphyrin with surrounding groups.[8] Further, the substrates to be oxidised are water soluble and would not be expected to appear in the hydrophobic core of the polymer. Thus, for reaction to occur, an edge or edges of the porphyrin must be available for electron transfer reactions at the junction of the outer hydrophilic and inner hydrophobic surfaces of the polymer. If this is the case then the oxidation reactions catalysed by the PSSS-VN–porphyrin system would be closely similar to those typical of natural peroxidases and might be expected to have similar activity.

One difference between the natural enzymes and the PSSS-VN system lies in the absence of a formal apical ligand to the metal centre in the porphyrin in the latter. In horse radish peroxidase, this function is served by an imidazolyl nitrogen atom.[1b] In the PSSS-VN system at pH 8, the only apical ligand is likely to be H_2O or OH^-. As catalytic activity in metalloporphyrins is influenced by the nature of such apical ligands, the activities of the horse radish peroxidase and the PSSS-VN–porphyrin mimic might not be expected to be identical. However, it has been demonstrated for oxidising systems with simple porphyrins as catalysts that the easily oxidised imidazole ligand can be profitably replaced by other ligands,[15a,b] including non-oxidisable inorganic varieties.[15a] It is encouraging that the rates of oxidation in the two systems are closely similar and that the porphyrin in the PSSS-VN polymer is not rapidly destroyed through auto-oxidation. In other work to be reported elsewhere, it is shown that a catalytic system, in which a porphyrin is covalently bonded between naphthalene residues in an analogue of PSSS-VN polymers, also behaves similarly and that even very simple sandwich molecules having a porphyrin covalently bonded between aromatic residues mimic the action of a peroxidase. It is concluded that porphyrins held in PSSS-VN amphiphilic polymers mimic peroxidase enzymic activity and do not behave like most other model porphyrin enzyme mimics, which resemble P450 cytochromes.

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* E-mail: rj05@liv.ac.uk

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