Proc. Indian Acad. Sci. (*Chem. Sci.*), Vol. 113, No. 1, February 2001, pp 1–9

Peroxidase-like catalytic activities of ionic metalloporphyrins supported on functionalised polystyrene surface

MIKKI V VINODU and M PADMANABHAN*
School of Chemical Sciences, Mahatma Gandhi University, Priyadarshini Hills, Kottayam 686 560, India
e-mail: mgu@md2.vsnl.net.in

MS received 1 June 2000; revised 8 August 2000

Abstract. Metalloderivatives of anionic tetrasulphonated tetraphenylporphyrin (MTPPS, M = Mn(III), Fe(III) and Co(III)) were synthesized and immobilized on cationically functionalised divinylbenzene(DVB)–crosslinked polystyrene(PS). These supported catalysts (PS-MTPPS) were found to exhibit peroxidase-like activity. The co-oxidation of 4-aminoantipyrine and phenol by H_2O_2 was attempted with these catalysts to mimic this enzyme function. The catalytic efficiency of all these immobilized MTPPS was found to be superior to the corresponding unsupported MTPPS in solution. The effect of the central metal ion of the porphyrin, *p*H of the reaction medium and also the temperature effect are investigated. The ideal *p*H was seen to be in the 8·0–8·5 range, with maximum effect at 8·2. The efficiency order for the various PS-MTPPS was seen to be Co>Mn>Fe, with CoTPPS showing efficiency comparable to that of horseradish peroxidase. The catalytic efficiency was found to be increasing with temperature for all the catalysts. The re-usability of these PS-MTPPS systems for peroxidase-like activity was also studied and it was found that they exhibited a very high degree of recyclability without much poisoning.

Keywords. Polystyrene support; metalloporphyrins; peroxidase model; enzyme catalysis.

1. Introduction

Peroxidases, which encompass a group of specific enzymes, are known for their vital role in biological systems by way of catalytically oxidising a wide variety of electron donor substrate species bearing phenolic, amine or acid functions aided by H_2O_2 or organic peroxides [1,2]. The best studied peroxidase is horseradish peroxidase (HRP), which is present in high concentration in the root of the horseradish plant. Others of interest are cytochrome *c*-peroxidase, chloroperoxidase, myeloperoxidase, lactoperoxidase, thyroid peroxidase and glutathione peroxidase. The core of these metalloenzymes is the highly tunable heme moiety, which is buried in protein envelopes. While a wide variety of model systems have been developed based on metalloporphyrins to mimic metalloenzymes [3–8], only a very few systems have been studied to model the enzymatic reaction of peroxidases [9–13]. We have been interested in developing polymer-bound metalloporphyrin-based catalysts and enzyme model systems [14–16]. While demonstrating the efficient catalase activity of solid-polymer supported metalloporphyrins, we reported

*For correspondence

the enhanced activity, increased stability, easy separation of the catalysts from the products and high degree of recyclability [14]. Inspired by this we have now investigated the peroxidase-like activity of some of the polymer-immobilized metalloporphyrins. We discuss here some of the factors contributing to the efficiency of the enzymatic reactions of ionic Fe(III), Mn(III) and Co(III) porphyrins immobilised on a cationically functionalised polystyrene support.

2. Experimental

2.1 *Preparative details*

The synthesis and characterisation of tetrasulphonated tetraphenylporphyrin (H_2TPPS) and its metalloderivatives MTPPS (M = Fe(III), Mn(III) and (Co(III)) were done according to the reported procedure [17–19]. The polymer support (PS) was prepared by functionalising 2% divinylbenzene (DVB) crosslinked polystyrene beads with chloromethylether followed by the reaction with pyridine as described in our earlier work [14]. The anionic MTPPS moieties were immobilized on the cationic PS thus obtained, by simple ion exchange method in aqueous conditions to get PS-MTPPS. The extent of porphyrin uptake on polymer surface was estimated to be 0·1 meq of porphyrin/g of polymer.

2.2 *Monitoring of peroxidase-like activity*

The peroxidase-like activity of the immobilised MTPPS (PS-MnTPPS, PS-FeTPPS and PS-CoTPPS) was monitored through dye formation reaction between 4-aminoantipyrine and phenol in presence of H_2O_2. In a typical reaction 12·5 mg of the polymer-bound catalyst (containing $1{\cdot}25 \times 10^{-6}$ mole MTPPS) was added to 5 ml of 1:1:3 mixture (v/v) of phenol ($0{\cdot}75 \times 10^{-4}$ mole/ml), 4-aminoantipyrine ($0{\cdot}25 \times 10^{-5}$ mole/ml) and the buffer solution. The buffers used were of the *p*H range 1·0 to 13·0. Hydrogen peroxide (1 ml, $0{\cdot}176 \times 10^{-2}$ N) was added to this and stirred slowly for a specified time. The absorbance of the solution was then measured at 505 nm (l_{max} of quinoid dye) against the reagent blank. The relative activities of the PS-MTPPS resins were then evaluated by comparing the absorbance of the quinoid dye formed with the value obtained in the case of HRP under the same conditions as reported previously [10].

2.3 *Physical measurements*

Spectral and absorption measurements were carried out by using a Shimadzu UV-160A spectrophotometer. Electronic spectra (in solution) of the porphyrins were measured either in water or in methanol. Solid state electronic spectra of the porphyrins and supported porphyrins were measured by grinding the solid samples with nujol and uniformly spreading the fine paste obtained on a strip of Whatmann 41 filter paper. The reference strip contained paste made from the porphyrin-free polymer support.

3. Results and discussion

Peroxidase activities are manifested in a variety of forms in biological systems. In the present study we have considered the co-oxidation involving 4-aminoantipyrine, $AmNH_2$, (**1**) and phenol (PhOH) by H_2O_2 yielding antipyrilquinoneimine (**2**) as the peroxidase

model reaction developed by PS-MTPPS systems. The overall reaction can be summarized as in scheme 1.

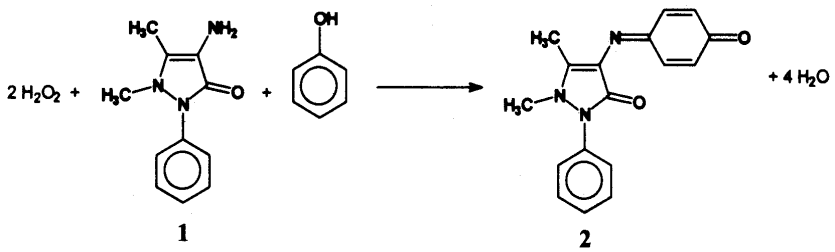
Compound **2** gives a characteristic absorption spectrum, with intense absorption bands and a l_{max} at 505 nm (e = 7870 M^{-1} cm^{-1}). This helped us to monitor the peroxidase-like reaction with ease using UV-VIS spectra.

The possible mechanisms of the chemical reactions involved in peroxidase reactions in biological systems have been established [2] which is summarized in scheme 2, where PFe^{3+} is the ferriheme moiety and DH_2 is the donor species.

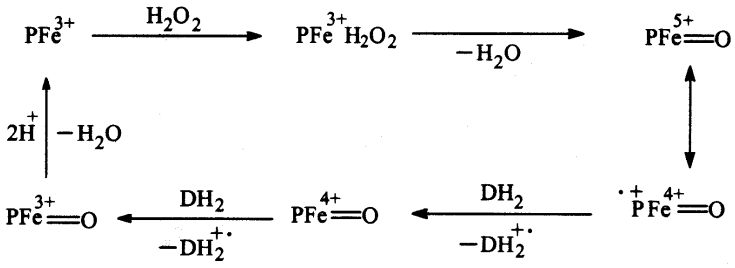
The two molecules of the electron donor species involved in the above reaction cycle can be of same or dissimilar type. In peroxidase enzymes the radical species generated could be scavenged by radical pairing and/or through other mechanisms possible in biological conditions. In the present peroxidase model reaction (scheme 1) the radical species that could be generated are $AmNH^{\bullet}$ and $PhO^{\bullet}$ (from **1** and PhOH respectively) [20]. As proposed by Metelitza *et al* [20] the recombination of these two radical species followed by stoichiometric oxidation through a second molecule of H_2O_2 can be assumed to complete the reaction pathways yielding the co-oxidised product **2**.

Considering the mechanisms involved in both catalase [14,21] and peroxidase, the first step involving the uptake of H_2O_2 by porphyrin to form a hypervalent metal-oxo species (Fe^{5+}=O) is seen to be common for both. While this reactive intermediate acts on and directly dismutates a second molecule of H_2O_2 in catalase, in peroxidase it acts on two donor DH_2 species successively, getting back to the initial state. This results in two different competitive reaction pathways as shown in scheme 3.

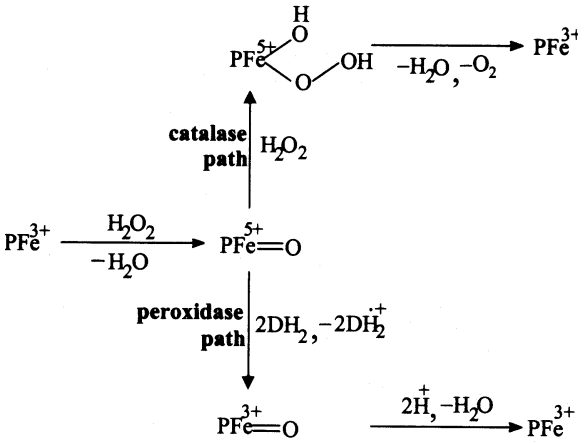
Thus, along with the peroxidase model reaction mentioned above, there is possibility of a competing catalase reaction also. While in the catalase path the (PFe^{5+}=O) formed



Scheme 1.



Scheme 2.



Scheme 3.

gets attacked by a second molecule of H_2O_2, in the peroxidase path the intermediate acts on two DH_2 moieties successively without any interference from the H_2O_2 in the catalytic site. In the present peroxidase model studies the uptake of the second H_2O_2 is obviously outside the porphyrin-centred catalytic site [20]. Since the rate of catalase reaction is faster than that of peroxidase [2], addition of H_2O_2 in the initial stage would preferentially lead to the catalase path. So to minimise the complication that may arise from the competitive catalase path we have used only an optimum amount of H_2O_2 which is preferably added as the last reagent in the reaction mixture.

Before investigating the activities of the solid PS-MTPPS, the peroxidase-like activity of the unsupported and water-soluble MTPPS (M = Fe^{III}, Mn^{III} and Co^{III}) were monitored for evaluating the relative efficiencies of the homogeneous and heterogeneous species. As expected, the monitoring of the peroxidase-like reaction with the soluble catalysts (MTPPS) gave difficulties as there was absorption overlap of the porphyrin moieties with that of the antipyrilquinoneimine (**2**) formed. However, we tried to circumvent this as much as possible by using a blank solution (reference) containing the same amount of MTPPS, 4-aminoantipyrine and phenol without H_2O_2. The soluble MTPPS are seen to exhibit a moderate peroxidase-like activity to the extent shown in table 1. Among the three soluble MTPPS studied, the enzyme-like activity exhibited by FeTPPS is seen to be not as much as that showed by MnTPPS and CoTPPS. An important factor contributing to this trend could be the high possibility of *m*-oxo dimer formation for FeTPPS as compared to its Mn and Co analogues. The *m*-oxo dimer is known to be catalytically inactive [21] because of its inability to form the crucial intermediate.

Unlike unsupported metalloporphyrins, the polymer-grafted MTPPS showed promising results. Divinylbenzene cross-linked chloromethylated polystyrene beads after functionalisation with pyridine were employed as the polymer support in this work. As discussed in our earlier paper, the anionic MTPPS moieties were immobilised on the surface of the cationic *N*-alkylpyridinium functionalised polymer support (PS) as generated above by simple anion exchange method in aqueous condition to get various PS-MTPPS systems [14]. Appending of porphyrins on the polymer support was confirmed by the conspicuous colour change of the polymer beads and also by electronic spectral

Table 1. The relative efficiencies of various metalloporphyrins for the peroxidase-like reaction (*p*H 8·2, time 60 min).

Catalyst systems	Absorbance at 505 nm	Amount of **2** formed ($\times 10^{-6}$ mol)	Relative activity
HRP*	0·80	0·61	100
MnTPPS	0·35	0·26	44
CoTPPS	0·35	0·20	44
FeTPPS	0·25	0·19	31
PS-MnTPPS	0·56	0·43	70
PS-CoTPPS	0·79	0·59	98
PS-FeTPPS	0·45	0·34	56

* From ref. 10 (under identical experimental conditions)

measurements. Grafted MTPPS are not leachable from the polymer surface even at extreme *p*H conditions (*p*H-1·0 to 13·0) and in most of the common solvents, either polar or non-polar. An attempt was made to estimate the extent of the appended porphyrin on the support. This was carried out spectrophotometrically by measuring the absorbance decrement of the Soret peak of the MTPPS solution (of known concentration) after equilibrating with a known amount of solid PS. The estimation showed an uptake of 0·10 meq of MTPPS/g of resin. This low uptake value indicates that the immobilised porphyrin moieties are widely separated. The absorption maxima of polymer-supported metalloporphyrins were found to be shifted considerably to higher wavelength from that of the corresponding unsupported porphyrins, which indicated appreciable electronic modulation of porphyrin moieties by the polymer support.

In the case of these solid PS-MTPPS catalysts developed, reaction monitoring was easy and error-free as the polymer beads (bearing MTPPS) could be simply removed by filtration from the reaction mixture. For comparing the relative efficiencies we have maintained the same reaction conditions for both soluble MTPPS and supported PS-MTPPS systems. As shown in table 1, we find these polymer-supported systems to be very efficient peroxidase models, even comparable to the original HRP. The high degree of efficiency of the supported MTPPS could be attributed partially to the structural and electronic modifications brought about in the porphyrin system by the polymer network. In addition to this, the *m*-oxo dimerisation of MTPPS (especially for FeTPPS), which is one of the detrimental factors for enzyme activity, is also prevented in PS-MTPPS due to polymer immobilisation. In this context, it may also be noted that since the catalysts could easily be removed from the reaction mixture, the co-oxidised product (antipyrilquinoneimine) could be estimated spectrophotometrically without any interference from MTPPS absorption.

It is seen in our experiment that all the PS-MTPPS systems exhibit appreciable peroxidase-like activity but with varying efficiencies. At the optimum *p*H chosen (8·2), the PS-CoTPPS system was the most efficient while PS-FeTPPS was the least so. PS-MnTPPS showed intermediate activity. It is interesting to note that the Co species instead of the Fe (which is present in natural systems) exhibited the highest peroxidase-like activity in the synthetic porphyrin framework.

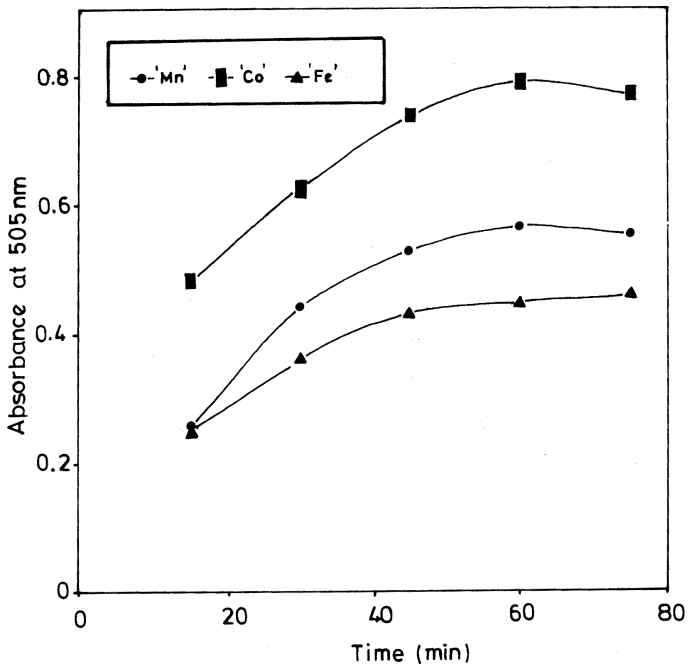


Figure 1. Yield of **2** against the incubation time (*p*H-8·2, temp. 30°C).

For all PS-MTPPS systems, the dye formation reaction was almost complete within 60 min as indicated in figure 1. Beyond this interval, some decrease in absorbance values (dye concentration) was observed for both PS-CoTPPS and PS-MnTPPS, which may be attributed to the physisorption of some of the dye formed onto the resin. This was physically verified.

Dependence of the *p*H on the formation of the product was monitored in the range 1·0–13·0, which showed remarkable variation in the enzyme-like reaction. Buffers employed in the present work to provide the *p*H required are varying concentrations of dil. H_2SO_4, acetate buffer, phosphate buffer, carbonate buffer and dil. NaOH. We find the maximum efficiency in the *p*H range 8·0 to 8·6 (peaking at 8·2). Above and below this narrow *p*H range, enzyme activity was seen to be negligible. The details are shown in figure 2.

At *p*H 8·2 the peroxidase activity shown by PS-CoTPPS reached about 95% of the activity exhibited by HRP under similar experimental conditions. In an earlier brief report [10] on MTPPS immobilised on amberlite resin the maximum efficiency was seen at *p*H 7·9. In that case MnTPPS showed the maximum activity as in the order MnTPPS> FeTPPS>CoTPPS. The observed difference in trend indicates the significant influence of the polymer matrix on enzyme tuning.

As reported previously by us, the above PS-MTPPS system exhibited maximum catalase-like activity around *p*H-10·0 in the dismutation of H_2O_2 [14]. Hence the observed difference in the optimal *p*H for catalase and peroxidase-like activities (10·0 and 8·2 respectively) suggests that the enzymatic activities of PS-MTPPS are very sensitive to *p*H variations and that they can be used selectively for different catalytic/enzyme actions by

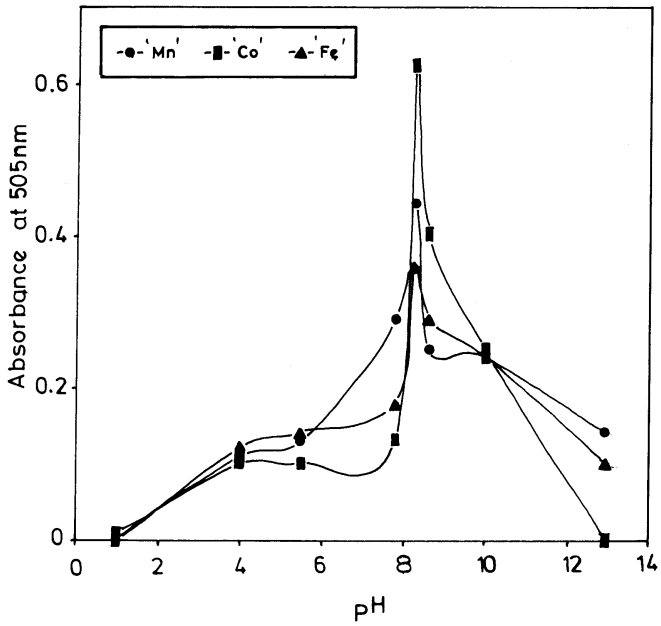


Figure 2. *p*H dependent yield of **2** (time 30 min, temp. 30°C).

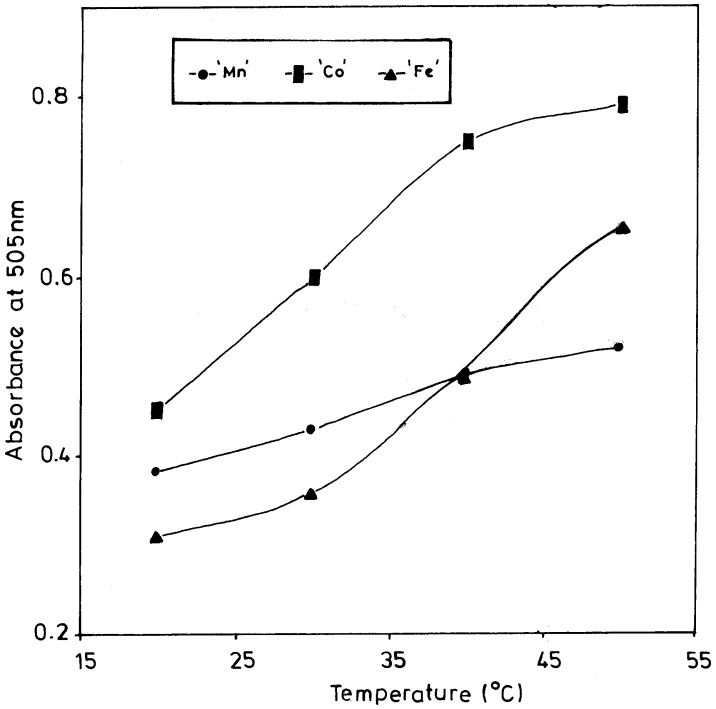


Figure 3. Temperature dependency on the peroxidase-like activities of PS-MTPPS systems (*p*H 8·2, time 30 min).

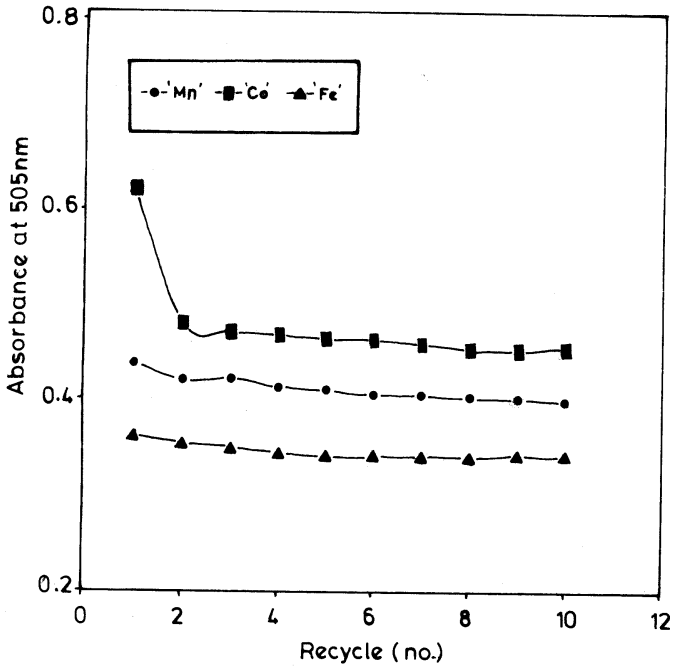


Figure 4. Recyclability of PS-MTPPS systems (temp. 30°C, time 30 min, *p*H 8·2).

choosing the appropriate *p*H. It is interesting to note that at *p*H 10, which is ideal for catalase activity, peroxidase-like activity is seen to be totally metal-independent, while catalase activity is found to be greatly metal-dependent (order being Mn>Co> Fe).

The effect of temperature on the peroxidase activity of PS-MTPPS system was also studied (between 20 and 50°C) maintaining the solution at *p*H 8·2 and keeping other reaction conditions the same. In all the cases, catalytic activity was found to be enhanced as temperature was increased (figure 3). However the PS-FeTPPS system exhibited more sensitivity to temperature variations than PS-MnTPPS or PS-CoTPPS. This is similar to their catalase activities and could be explained in terms of the possible temperature dependent spin cross-over which is dominant for Fe(III) complexes [14,22].

The reusability of the PS-MTPPS systems was also studied at *p*H 8·2 for 10 cycles. The PS-CoTPPS system showed some deterioration in activity after the first cycle, but no further decrease. We attribute this to the adsorption of some amount of the product formed onto the resin. On close examination of the beads we could verify this. Mn and Fe systems, however, exhibited higher degree of recyclability without much poisoning. However, the overall efficiency of the Co(III) system was found to be superior to that of Mn(III) and Fe(III) throughout the reaction cycle. The result is shown in figure 4.

This result confirms that PS-MTPPS systems behave as efficient catalysts and the metalloporphyrins are resistant to any oxidation by H_2O_2 when immobilised on polymer supports. Encouraged by this enhanced activity and increased stability of the PS-MTPPS catalysts, we are currently probing other related co-oxidation (peroxidase-like) reactions.

4. Conclusions

Polymer-supported metalloporphyrin systems developed in the present study were found to be more efficient catalysts for peroxidase-like activities than unsupported porphyrins. Catalytic activity was found to be highly metal and *p*H dependent and seen to be facilitated by increase in temperature of the reaction medium. All the catalysts were also found to be recyclable without much catalyst poisoning.

Acknowledgement

One of the authors (MVV) thanks the University Grants Commission, New Delhi for a fellowship.

References

1. Wirstam M, Blomberg M R R and Siegbahn P E M 1999 *J. Am. Chem. Soc*. **121** 10178
2. Likhtenshtein G I 1998 *Chemical physics of redox metalloenzyme catalysis* (Berlin, Heidelberg: Springer-Verlag)
3. Meunier B 1992 *Chem. Rev*. **92** 1411
4. Bedioui F, Devynck J and Biedcharreton C 1996 *J. Mol. Catal.* **113** 3
5. Lindsey J S 1994 In *Metalloporphyrin catalysed oxidations* (eds) F Montanari and L Casella (Amsterdam: Kluwer)
6. Matsui T, Ozaki S and Watanabe Y 1999 *J. Am. Chem. Soc.* **121** 9952
7. Bedioui F, Devynck J and Biedcharreton C 1995 *Acc. Chem. Res*. **28** 30
8. Fudickar W, Zimmermann J, Rahlmann L, Schneider J, Roder B, Siggel U and Fuhrhop J H 1999 *J. Am. Chem. Soc*. **121** 9539
9. Traylor T G, Lee W A and Stynes D V 1984 *J. Am. Chem. Soc*. **116** 755
10. Saito Y, Mifune M, Nakashima S, Tanaka Y, Chikuma M and Tanaka H 1986 *Chem. Pharmacol. Bull*. **34** 5016
11. Mao L, Yzhu M, Huang X M, Shen H X and Li R 1997 *Chem. J. Chin. Univ.* **18** 1611
12. Jones P, Mantle D and Wilson I 1983 *J. Chem. Soc., Dalton Trans*. 161
13. Labat G, Seris J L and Meunier B 1990 *Angew. Chem. Int. Engl*. **29** 1471
14. Vinodu M V and Padmanabhan M 1998 *Proc. Indian Acad. Sci. (Chem. Sci.)* **110** 461
15. Mathew T and Padmanabhan M 1996 *J. Appl. Poly. Sci*. **59** 23
16. Mathew T and Padmanabhan M 1995 *Indian J. Chem*. **B34** 903
17. Adler A D, Longo F R, Finarlly J D, Goldmacher J A and Korsakoff T 1967 *J. Org. Chem*. **32** 476
18. Srivastava T S and Tsutsui M 1973 *J. Org. Chem*. **38** 2103
19. Labat G and Meunier B 1989 *J. Org. Chem*. **54** 5008
20. Metelitza D I, Litvinchuk A V and Savenkova M I 1991 *J. Mol. Catal.* **67** 401
21. Pinnicucci R and Bruice T C 1990 *J. Am. Chem. Soc.* **122** 6003
22. Konig E 1991 *Struct. Bonding* **76** 51