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Synthetic porphyrins/metalloporphyrins which mimic states in catalytic cycle of cytochrome P-450 and peroxidases

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Abstract

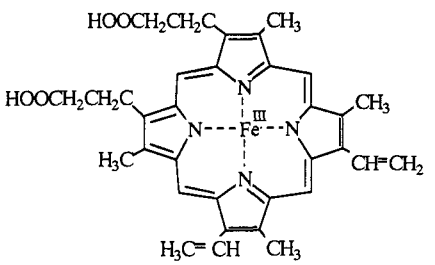
The reaction of thiolates, RS^-, having electron withdrawing groups with iron(III) tetra-arylporphyrins in DMSO,in air gives low-spin Fe(III)-P($^-$SR)$_2$, having hyperporphyrin spectra similar to thiolate derivatives of cytochrome P-450. Removal of air gives high spin Fe(II)-P($^-$SR) which on addition of CO results in H^+ uptake and formation of Fe(II)-P(SHR)CO. Addition of thiols and base to the iron(III) porphyrins in toluene in the absence of air gives 4-coordinate Fe(II)-P which in the presence of CO gives Fe(II)-P(SHR)CO.
Metal complexes of one of the porphyrins studied i.e. a tetraphenolic porphyrin undergo $2e^-$ oxidation in air to give quinonoid species which oxidise peroxidase substrates. Rate constants for some of these reactions are reported.

INTRODUCTION

Cytochrome P-450 are a group of haem containing enzymes which participate in several important biological processes. These include the biosynthesis and biodegradation of endogenous compounds such as steroids, fatty acids or prostaglandins, the detoxification of exogenous compounds such as drugs and environmental products by metabolic oxidation and the carcinogenic activation of polycyclic aromatic hydrocarbons (refs. 1 & 2). They are classified as monooxygenases since they catalyse the hydroxylation of substrates by the reductive activation of molecular oxygen, equation 1 (refs. 3 & 4). They can also catalyse the transfer of an oxygen atom to multiple bonds, and to heteroatoms of various substances.

$$RH + O_2 + 2e^- + 2H^+ \longrightarrow ROH + H_2O \qquad (1)$$

The prosthetic group of the cytochrome P-450 enzymes is iron(III)-protoporphyrin(IX), 1. The haem group is linked to the protein by a cysteinate residue (ref. 5) and in the resting state of the enzyme an equilibrium exists between a predominant (~94%) low spin 6-coordinate iron(III) form in which the sixth donor atom is most likely oxygen from H_2O, 2, and a high spin 5-coordinate iron(III) form, 3, (ref. 6). Binding of substrate occurs at a protein site close to the haem group and causes a shift in the equilibrium towards the high spin ferric state, the shift depending on the substrate (ref. 6).



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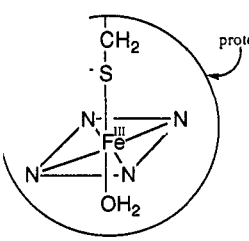

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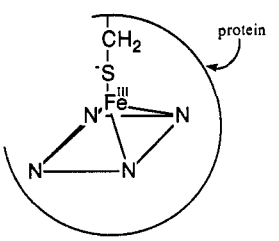

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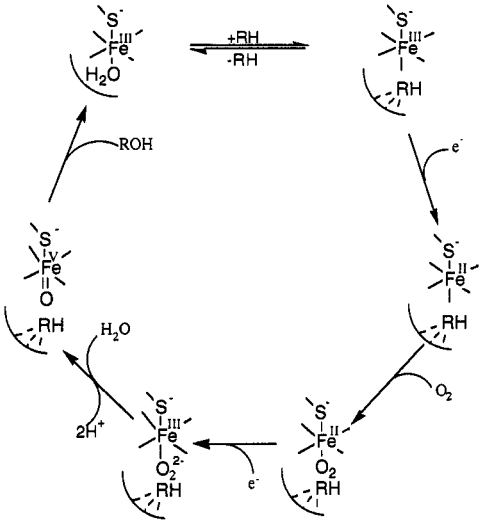


Fig. 1. Catalytic cycle of cytochrome P-450

The catalytic cycle of the P-450 enzymes involve substrate binding, reduction of the high spin substrate-bound form to iron(II) followed by oxygenation and a second 1 e⁻ reduction and protonation to give a species such as Fe(III) - O-OH from which the active oxidant is derived by heterolytic fission of the O-O bond (ref. 1), Fig. 1. Because of its extremely short lifetime the true nature of the active oxidant is unknown and various possible formulations such as an oxyperferryl porphyrin containing the Fe(V) = O group or an oxyferrylporphyrin cation radical (ref. 1) and others (ref. 7) have been proposed.

The cytochrome P-450 group of enzymes is so called because the addition of CO to the iron(II) form of the enzymes (obtained following reduction with dithionite) gives a derivative having an unusual absorption spectrum, classified as a hyperporphyrin spectrum in which the Soret band is split into 2 components at ~363 and 450 nm (refs. 8 & 9). The 450 nm band is frequently used in assays of the enzyme. Hyperporphyrin spectra have also been obtained for complexes of cytochrome P-450 with organic thiols (ref. 10) and the species responsible for the hyperporphyrin spectra in this case are low-spin bis(thiolato)iron(III) porphyrin complexes, one thiolate ligand being from the added thiol the other from the cysteinate of the protein (ref. 11). The splitting is due to the interaction of a cysteinate S to porphyrin π^* transition with the $\pi \longrightarrow \pi^*$ transition of the metalloporphyrin normally responsible for the Soret band.

REACTION OF THIOLATES WITH IRON(III) TETRAARYLPORPHYRINS

Previous attempts to mimic the spectra of cytochrome P-450/thiolate adducts at room temperature using synthetic iron(III) porphyrins and alkylthiolate ligands were unsuccessful because of the susceptibility to reduction of iron(III) to iron(II) under such conditions (ref. 12). This problem has been circumvented either by the use of low temperatures which prevents reduction (ref. 11) or as we have done by using thiols with electron withdrawing groups and synthetic tetraarylhaems at room temperature (ref. 13). Addition of Et_3N (0.16M) to a solution of methyl thioglycolate (0.05M) and tetraphenylporphyriniron(III) chloride (1.3 x 10^{-5}M), Fe(III)TPPCl in DMSO produced a hyperporphyrin spectrum, Fig. 2, which on the basis of comparison with the enzyme derivative we have assigned to the low-spin bis(thiolato)iron(III) porphyrin, 4. The magnetic moment of this species in DMSO solution (1.58 x 10^{-3} g/cm^{-3}) was determined by the Evans NMR method (ref. 14) by measuring the shift in the C-H t-butanol signal caused by the paramagnetic species and found to be 2.13 BM thus confirming that it is indeed low spin iron(III) with one unpaired electron for which μ_S is 1.73 BM (ref. 15). This species is stable in air at room temperature. Similar hyperporphyrin spectra were obtained for other thiols under the same conditions provided the thiol has electron withdrawing groups e.g. $SHCH_2CO_2H$, SHC_6H_5, SHC_6F_5 (ref. 13). With thiols not having electron withdrawing groups e.g. simple alkylthiols decomposition of the haem occurred presumably following reduction of the iron(III). Haemins derived from other porphyrins, having substituents on the aryl rings and on the tetrapyrrolic periphery, 5, also gave hyperporphyrin spectra.

$$\begin{array}{l} {}^{-}SCH_2CO_2Me \\ | \\ Fe^{III}(TPP) \\ | \\ {}^{-}SCH_2CO_2Me \end{array}$$

4

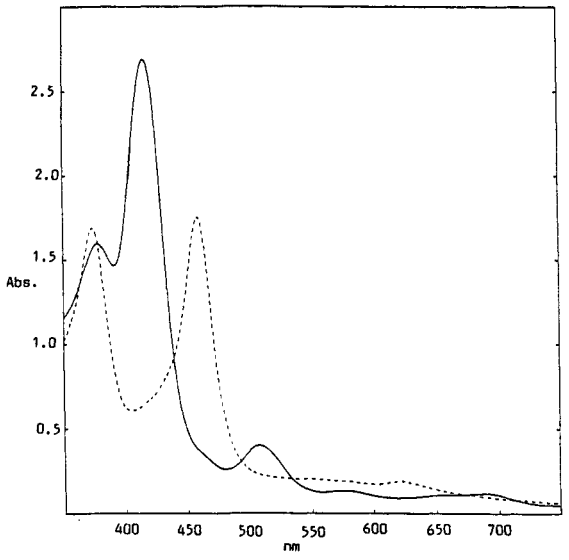
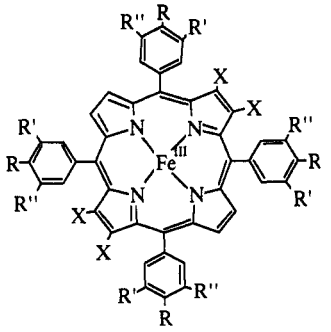


Fig. 2. Electronic spectra of Fe^{III} (TPP)Cl in DMSO before (solid line) and after (dashed) the addition of $SHCH_2CO_2Me + Et_3N$.



	R	R'	R''	X
(a)	H	H	H	H
(b)	H	H	OMe	H
(c)	H	OMe	OMe	H
(d)	OMe	H	H	H
(e)	OH	H	H	H
(f)	H	H	H	Br
(g)	OH	Bu^t	Bu^t	H

5

When the solution containing the species responsible for the hyperporphyrin spectrum was deaerated by passing nitrogen through it gradual reduction of iron(III) to iron(II) occurred giving a species having an electronic spectrum characteristic of a high spin 5-coordinate thiolatoiron(II)-tetra-arylporphyrin complex, Fig. 3. The magnetic moment in DMSO solution (3.62×10^{-4} g/cm^3) obtained by the Evans NMR method was 5.34 BM corresponding to 4 unpaired electrons per iron (μ_S = 4.90 BM) and indicative of high spin iron(II), 6. This reaction was reversed by the addition of air and this aeration/deaeration sequence could be repeated indefinitely without haem decomposition. Addition of CO to the thiolatoiron(II)-porphyrin solution gives an adduct, 7, with a normal Soret absorption band at 420 nm, Fig. 4. This is not a CO adduct of a thiolatoiron(II)

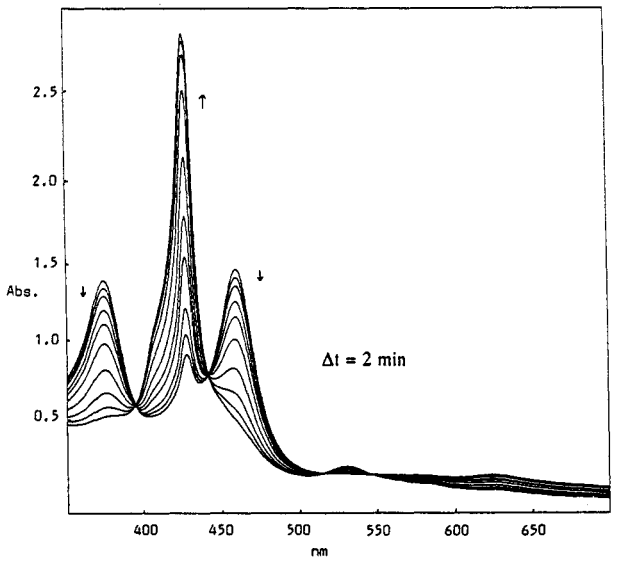


Fig. 3. Spectral changes in the conversion of Fe(III)TPP($^-SCH_2CO_2Me)_2$, 4, ⟶ Fe(II)TPP($^-SCH_2CO_2Me$), 6, in DMSO.

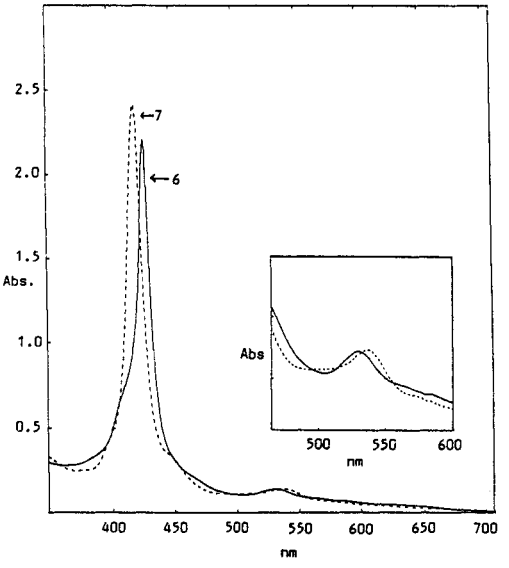
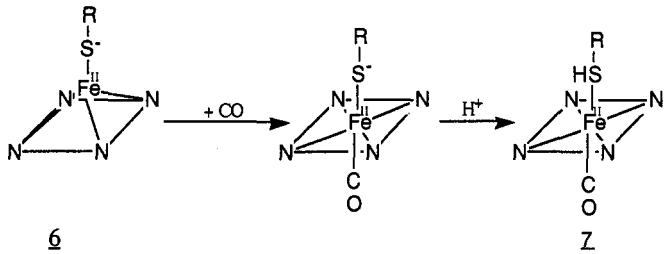


Fig. 4. Spectral changes in the conversion of Fe(II)TPP($^-SCH_2CO_2Me$), 6, ⟶ Fe(II)TPP($HSCH_2CO_2Me$)CO, 7.



6 7

Fig. 5. Formation of 7 in DMSO.

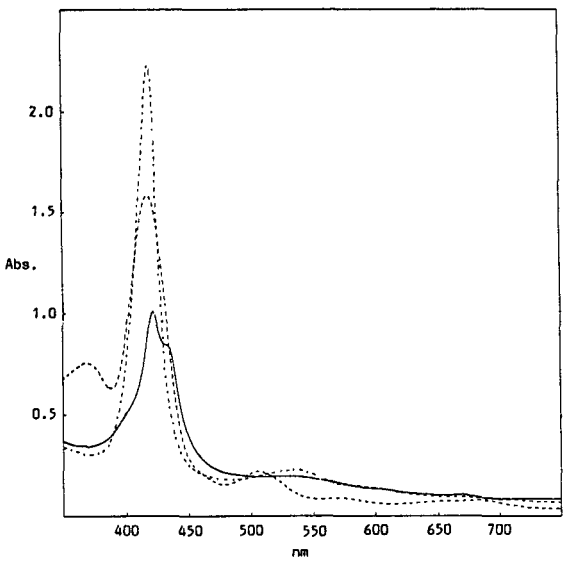
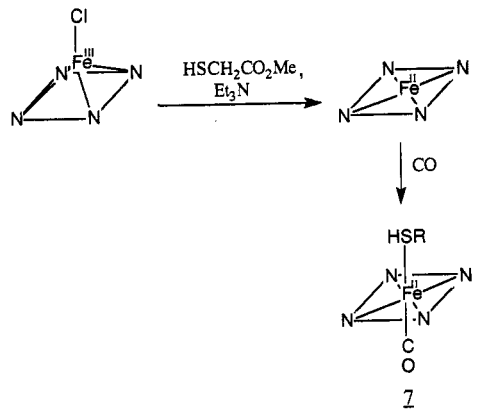


Fig. 6. Spectra of Fe(III)TPPCl (--), Fe(II)TPP (solid line) and Fe(II)TPP(SHCH$_2$CO$_2$Me)CO (-·)in toluene.



7

Fig. 7. Formation of 7 in toluene.

porphyrin since this would produce a hyperporphyrin spectrum. The product is likely to be a low spin CO adduct of a thioliron(II) porphyrin, which would have a normal Soret band (ref. 16), resulting from H^+ uptake by the thiolate ligand. Proton uptake by the thiolate ligand on the addition of CO may be due to the increased basicity as a result of movement of iron(II) into the porphyrin plane. In the high spin 5-coordinate iron(II) complex, 6, the iron sits over the porphyrin plane towards the thiolate, Fig. 5. This means that there is less σ donation from the porphyrin and more from the thiolate. Addition of CO causes the metal to move into the porphyrin plane with concomitant lengthening of the Fe-S bond. The porphyrin in this situation is a better σ donor and less electron density is removed from the thiolate which enhances its basicity and causes uptake of a proton from the medium giving 7.

Addition of Et_3N to a solution of methylthioglycollate and tetraphenylporphyriniron(III) chloride in toluene in the presence of air did not produce a hyperporphyrin spectrum. However under nitrogen reduction of iron(III) to iron(II) occurred with the formation of the previously characterised (ref. 17) 4- coordinate Fe(II)TPP, Fig. 6. Addition of CO to this solution gave the 6-coordinate Fe-TPP(CO)(SHCH$_2$CO$_2$Me), 7. It appears therefore that the thiol only coordinates to the iron(II) porphyrin if CO is present. The fact that the thiol ligand does not bind to square planar Fe(II)-TPP is because it is a weak σ donor and because of repulsion by electrons in the d_z^2 orbital of the metal, Fig. 7. Adduct formation with CO however causes back bonding from the metal to this ligand, increases its Lewis acidity and allows coordination of the thiol.

The reaction sequences in DMSO and toluene are shown in Fig. 8.

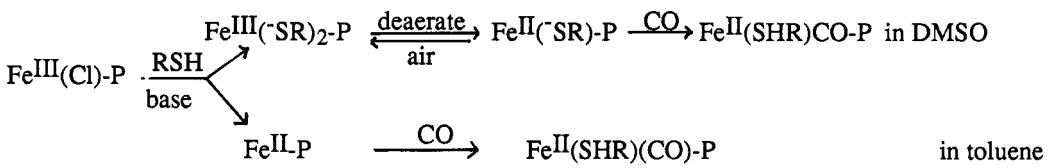


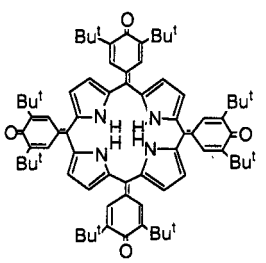
Figure 8

METALLOPHENOLIC PORPHYRINS

The porphyrin in structure 5(g) having phenolic substituents was originally synthesised to mimic Compound I of the peroxidases. The peroxidases are haem proteins which catalyse the oxidation of substrates by hydrogen peroxide or by alkyl or acyl hydroperoxides (ref. 18). Like cytochrome P-450 the haem group of most peroxidases is iron(III)- protoporphyrin IX and in the catalytic cycle this is oxidised by the peroxide to an oxyferrylporphyrin cation radical, i.e. Compound I which is reduced by substrate in two 1 e⁻ steps producing another intermediate, Compound 2 on the way although there are exceptions in which the production of Compound 2 is bypassed.

Using simple iron porphyrins to mimic Compound I has met with difficulty because attempts to oxidise these at room temperature in order to get porphyrin radicals generally cause decomposition of the haem. A number of different approaches have been used in the past to circumvent this problem. This has included the use of haems derived from tetra-arylporphyrins having alkyl substituents on the phenyl rings (refs. 19 & 20) or perhalogenated porphyrins having halogen substituents on the tetrapyrrolic periphery or on the phenyl ring (refs. 21 & 22). Some time ago porphyrins with 3,5-di-t-butyl-4-hydroxyphenyl substituents in the meso position were synthesised in the hope that the phenolic groups would accommodate radical formation and that the t-butyl groups present would protect the radical so formed from intermolecular association as is the case in the stabilisation of the tri-t-butylphenoxy radical (refs. 23 & 24).

This porphyrin, TBHPP, and some of its metal complexes undergo oxidation by m-chloroperbenzoic acid (spectrophotometric titration shows it to be a 2 e⁻ oxidation) or by air in basic solution which results in the same product (ref. 23). The oxidation product of the porphyrin has been isolated in crystalline form and shown to be a porphyrinogen, 8, (ref. 25) having coplanar quinonemethide groups and a highly puckered macrocyclic core. While some metal complexes of the porphyrin e.g. Zn(II), Ni(II), Fe(III) were found to undergo facile aerial oxidation others such as the Pd(II) complex were resistant to such oxidation (ref. 26). This has previously been ascribed to a structural effect based on crystal structure determinations of the Ni(II) and Pd(II) complex. In the palladium(II)-porphyrin complex the metal-nitrogen bond distance is 2.022 Å which is close to the ideal porphyrin hole radius and allows the porphyrin to be planar. The smaller ionic radius of nickel(II) however results in a metal-nitrogen bond distance of 1.91 Å which causes the porphyrin core to pucker. This permits interaction between the π clouds of the aryl rings in the meso positions with that of the porphyrin which facilitates aerial oxidation and formation of metal complexes of the oxidised porphyrin. The structures of the oxidised metalloporphyrins have so far not been determined. However all the oxidised metal complexes investigated i.e. Zn(II), Fe(III), Ni(II), have very similar electronic spectra, Fig. 9, indicating similar sites of oxidation and since in one of these i.e. the zinc(II) complex, the metal cannot be oxidised it follows that in all cases 2e⁻ oxidation of the porphyrin occurs. A possible structure for the oxidised metalloporphyrins is shown in 9 although it may also be a complex of the tetraanion of porphyrinogen 8 (ref. 27).



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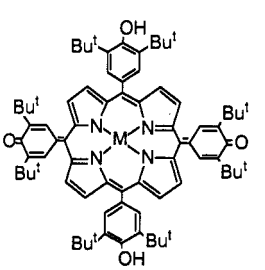

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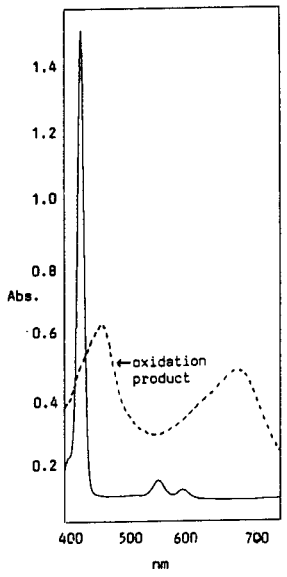

Fig. 9. Spectra of Zn(II)-TBHPP and its 2e- oxidation product in chloroform.

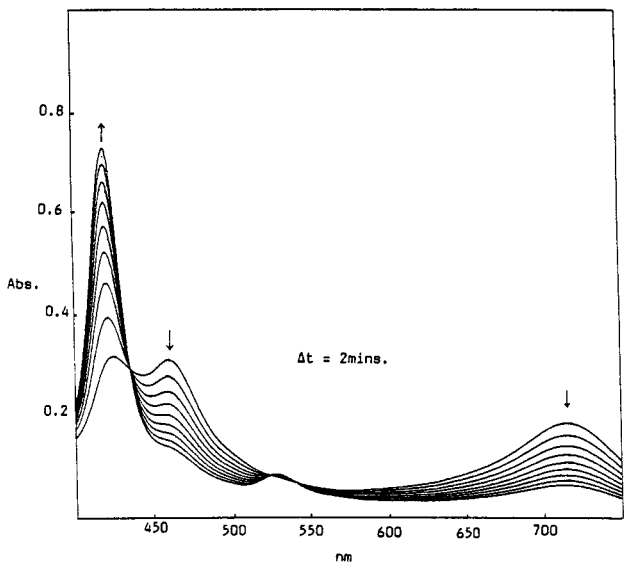

Fig. 10. Spectral changes accompanying the reduction of oxidised Ni(II)-TBHPP by p-cresol (2M) in methanol.

The oxidised metalloporphyrins react with peroxidase substrates such as p-cresol and are reduced cleanly and quantitatively to the parent metalloporphyrin, Fig. 10. We have studied the kinetics of these reactions spectrophotometrically by monitoring the reappearance of the Soret band of the metalloporphyrin both in chloroform and methanol solution under pseudo first order conditions using a vast excess of p-cresol. A stock solution of each oxidised metalloporphyrin was prepared by treating a solution of the metalloporphyrin in chloroform ($\sim 10^{-4}$M) with a 40% solution of tetrabutyl ammonium hydroxide in water ($2cm^3$). The solution was washed several times with equal volumes of water and dried over sodium sulphate. A small quantity of this solution was then injected into the reaction solution containing p-cresol.

Values of k_{obs} at various p-cresol concentrations at 298K are presented in Table 1. For the reduction of the oxidised metalloporphyrins, with the exception of the nickel(II) porphyrins in chloroform, k_{obs} was found to vary linearly with p-cresol concentration, equation 2.

$$\text{rate} = \{k_1 + k_2[\text{p-cresol}]\}[\text{M-por}_{ox}] \qquad (2)$$

This rate law implies that two reduction pathways operate, the k_1 pathway involves auto or solvent assisted reduction of the oxidised metalloporphyrin, the other involving reduction by p-cresol. In the case of the oxidised nickel(II) porphyrin complex in chloroform k_{obs} was found to vary linearly with the square of the p-cresol concentration, equation 3.

$$\text{rate} = \{k_1 + k_3[\text{p-cresol}]^2\}[\text{M-por}_{ox}] \qquad (3)$$

The second order dependence of rate on p-cresol concentration we attribute to pre-equilibrium formation of a H-bonded p-cresol dimer which is the active reductant in this solvent. Values of k_1, k_2 and k_3 obtained from the slopes and intercepts of the above plots are shown in Table 2.

Solvent effects on rates of reaction could only be assessed in the case of the iron(III) porphyrin complex since in the case of the zinc(II) complex reproducible kinetic data could not be obtained in chloroform whereas in the case of nickel(II) complex different rate laws apply in chloroform and methanol. The reduction of the oxidised iron(III) porphyrin in methanol is 2-3 times faster than in chloroform. For the three complexes studies the order of reactivity in methanol was found to be

$$\text{Fe(III)} \sim \text{Zn(II)} \gg \text{Ni(II)}$$

The effect of temperature on reaction rate was investigated in the case of the nickel(II) complex. From the Arrhenius plots of ln k_2 vs 1/T (methanol as solvent) and ln k_3 vs 1/T (in chloroform) the following activation parameters were calculated for 298K;

in methanol $\Delta H^{\#} = 67.3 \pm 2.1$ kJ mol^{-1} $\Delta S^{\#} = -77 \pm 8$ J K^{-1} mol^{-1}

in chloroform $\Delta H^{\#} = 67.9 \pm 2.1$ kJ mol^{-1} $\Delta S^{\#} = -77 \pm 8$ J K^{-1} mol^{-1}

Table 1. Values of k_{obs} for the reduction of the oxidised metalloporphyrin complexes by p-cresol in chloroform and methanol at 298K. All values are ± 5%.

Complex	Chloroform [p-cresol] mol dm^{-3}	Chloroform k_{obs}/s^{-1}	Methanol [p-cresol] mol dm^{-3}	Methanol k_{obs}/s^{-1}
Ni(II) TBHPP	2.0	3.10×10^{-3}	2.0	1.93×10^{-3}
	1.5	1.68×10^{-3}	1.5	1.53×10^{-3}
	1.0	7.56×10^{-4}	1.0	1.05×10^{-3}
	0.5	2.59×10^{-4}	1.0	5.56×10^{-4}
	0.25	8.77×10^{-5}	0.25	3.22×10^{-4}
Fe(III)TPHPP	0.25	2.64×10^{-2}	0.15	4.53×10^{-2}
	0.15	1.54×10^{-2}	0.10	2.63×10^{-2}
	0.05	4.35×10^{-3}	0.05	9.07×10^{-3}
	0.025	1.99×10^{-3}	0.03	5.21×10^{-3}
Zn(III)TBHPP*			0.15	2.06×10^{-2}
			0.10	1.41×10^{-2}
			0.05	9.88×10^{-3}
			0.025	5.70×10^{-3}

* Reproducible kinetic data could not be obtained for the reduction of this complex in chloroform.

Table 2. Rate constants for the reduction of oxidised metalloporphyrins by p-cresol at 298K.

Complex	Chloroform k_1/s^{-1}	Chloroform k_2/dm^{3}mol^{-1}s^{-1} or k_3/dm^{6}mol^{-2}s^{-1}	Methanol k_1/s^{-1}	Methanol k_2/dm^{3}mol^{-1}s^{-1}
Ni(II)-TBHPP	3.04×10^{-5}	7.58×10^{-4} (k_3)	9.95×10^{-5}	9.31×10^{-4}
Fe(III)-TBHPP	-	1.09×10^{-1} (k_2)	-	2.40×10^{-1}
Zn(II)-TBHPP	-	-	3.31×10^{-3}	1.14×10^{-1}

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