An Electron Spin Resonance Study of Frozen Aqueous Solutions containing 5,10,15,20-Tetrakis(*N*-methyl-4'-pyridinio)porphyrinatocobalt(II)

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E.s.r. spectra are reported for frozen solutions (77 K) of the 5,10,15,20-tetrakis(*N*-methyl-4'-pyridinio)porphyrinatocobalt(II) cation in pure water and water containing a variety of N donors. In pure water the (hydrated) monomer seems to be present, but with most of the N donors [including piperazine-*N*,*N*'-bis(ethane-2-sulphonic acid) buffer], N hyperfine splitting in the e.s.r. spectra shows the formation of 1 : 1 adducts. The reactivity of these species towards dioxygen has been investigated, and a number of dioxygen adducts characterized.

There have been a number of studies of the e.s.r. spectra, in frozen glassy media, of Co^{II} porphyrin complexes, their adducts with N donors, and the reaction products of these adducts with dioxygen.[1-4] In the presence of N donors L, the ^{14}N hyperfine splitting in the e.s.r. spectra shows the initial formation of 1 : 1 complexes and also, with an excess of the better donors, 2 : 1 complexes containing six-co-ordinate Co^{II}. These adducts react with O_2 to give products with very different e.s.r. spectra, which contain one ligand and one oxygen molecule, and are best formulated as $[Co^{III}L(O_2)P]$ (P = a porphyrinate). At low temperatures, the uptake of oxygen is normally reversible, but at room temperature a further reaction occurs to give e.s.r. silent species of the general formula $[(L)PCo^{III}(O_2)Co^{III}P(L)]$. The original Co^{II} adducts can often be regenerated by deoxygenation of these species, usually at higher temperatures.

The majority of the Co^{II} porphyrin complexes which have been investigated in this way are insoluble in water, the normal biological medium. Smith and co-workers[5] studied the water-soluble complex [5,10,15,20-tetrakis(*p*-sulphonatophenyl)porphyrinato]cobaltate(II), $[Co^{II}(tspp)]^{4-}$. However, it is well known that H_2tspp^{4-} and its metal complexes aggregate strongly in aqueous solution.[6] No e.s.r. spectrum was obtained from solutions of $[Co^{II}(tspp)]^{4-}$ in pure water and it was necessary to add organic solvents such as dimethylformamide or dimethyl sulphoxide to cause disaggregation of the metalloporphyrin,[5] with the consequent possibility of co-ordination by the organic component. E.s.r. spectra characteristic of monomeric species have, however, been observed for frozen aqueous solutions of Co^{II}-substituted myoglobin and haemoglobin, and their O_2 adducts.[7] There is evidence[6,8,9] that metal complexes of 5,10,15,20-tetrakis(*N*-methyl-4'-pyridinio)porphyrin (H_2tmpyp^{4+}) do not aggregate in aqueous solution, and we have accordingly studied the e.s.r. spectra, in frozen aqueous solution at 77 K, of systems containing, or derived from, the Co^{II} complex of this ligand.

Experimental

The porphyrin H_2tmpyp^{4+} was prepared, as its tetra(toluene-*p*-sulphonate) salt, by the method of Pasternak *et al.*[9] (Found: C, 63.10; H, 4.85; N, 8.20. $C_{72}H_{66}N_8O_{12}S_4$ requires C, 63.40; H, 4.90; N, 8.20%). Solutions of $[Co^{II}(tmpyp)]^{4+}$ were obtained by heating $Co(NO_3)_2 \cdot 6H_2O$ and a *ca.* 10% excess of H_2tmpyp^{4+} in water at 80 °C for 24 h in an Ar atmosphere. The concentrations varied from 2×10^{-3} to 2×10^{-2} mol dm^{-3}, with the more dilute solutions giving better resolved e.s.r. spectra. The formation of the complex was conveniently studied by measuring the magnetic moment μ of Co^{II} using an n.m.r. method.[10] After *ca.* 24 h at 80 °C, μ became constant at 2.10 B.M., which is characteristic of low-spin Co^{II}. Except where deliberate exposure to O_2 was required, solutions were handled in an Ar atmosphere.

Benzimidazole (Aldrich) was twice sublimed in vacuum, and piperazine-*N*,*N*'-bis(ethane-2-sulphonic acid) (pipes, Sigma) was recrystallized from H_2O and dried in vacuum. A buffered solution of $^{15}NH_3$ was prepared by adding 99.8% $^{15}NH_4NO_3$ (0.04 g) (Prochem) to 0.25 mol dm^{-3} NaOH (1 cm^3). All other ligands were used as received. Unless otherwise stated, the ligand concentration in the e.s.r. experiments was 0.05 mol dm^{-3}.

The e.s.r. spectra were measured at 77 K on a Varian E12 spectrometer operating at *ca.* 9.2 MHz. The addition of ethane-1,2-diol or glycerol (10—20% v/v) to the aqueous solutions before rapid freezing sometimes gave better resolution. Since it also caused slight changes in the e.s.r. spectra, this technique was not normally adopted. The e.s.r. spectra were analysed to obtain *g* and *A* values by standard techniques.[1,2]

Results and Discussion

Two complications were found in the e.s.r. spectra. First, a sharp resonance at $g \sim 2.00$, which was also observed with the free porphyrin. Similar signals have been reported for other porphyrins[11] and attributed to a free-radical species. Secondly, at high gain a multi-line spectrum could be seen in this region. A study of pure $[Cu^{II}(tmpyp)]^{4+}$ showed that this arose from traces of Cu^{II} present as an impurity (*cf.* Assour[11]).

$[Co^{II}(tmpyp)]^{4+}$ *in the Absence of Added Ligands.*—The e.s.r. spectrum of $[Co^{II}(tmpyp)]^{4+}$ in frozen aqueous solution at 77 K is shown in Figure 1(*a*), and can be assigned to the monomeric form with one or two H_2O molecules co-ordinated axially, which will be represented as CoP(O).

The spectrum is very similar to that observed by Smith and co-workers[5] for $[Co^{II}(tpps)]^{4-}$ in H_2O–dimethylformamide at 77 K, and thought to arise from a six-co-ordinate Co^{II} species. When O_2 was bubbled through an aqueous solution of $[Co^{II}(tmpyp)]^{4+}$ at room temperature for 3 min, and the e.s.r. tube immediately cooled in liquid N_2, the spectrum shown in Figure 1(*b*) is obtained. The CoP(O)-type spectrum has almost completely disappeared and has been replaced by one characteristic of an O_2 adduct, $CoP(O)(O_2)$. Although there is no direct evidence, it seems likely that a water molecule is co-ordinated *trans* to the oxygen, which will increase the electron density on the Co in the same manner as a N base in the organic-soluble Co^{II} porphyrin systems.[1,2] Purging the thawed solution with Ar overnight (room temperature) regenerated the CoP(O)-type spectrum at 77 K with *ca.* 25% of its original intensity.

If O_2 was bubbled through the solution of $[Co^{II}(tmpyp)]^{4+}$ in water (or a 0.05 mol dm^{-3} succinate buffer at pH 6) for 1 h, only

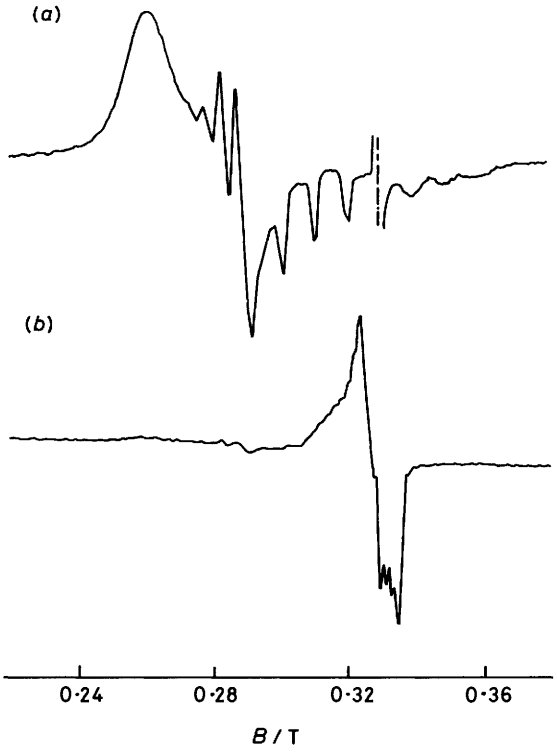

Figure 1. The e.s.r. spectra at 77 K of (*a*) $[Co(tmpyp)]^{4+}$ in water and (*b*) the same solution after O_2 has been bubbled through for *ca.* 3 min at room temperature before cooling

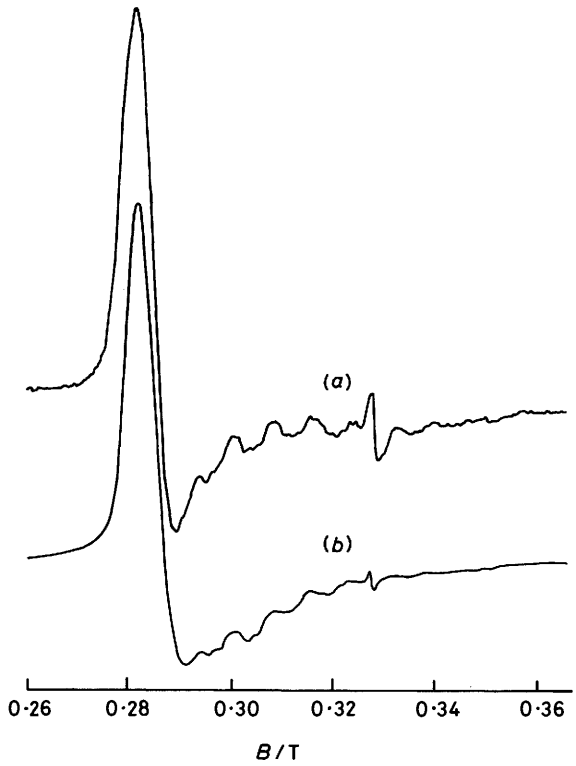

Figure 2. The e.s.r. spectra at 77 K of $[Co(tmpyp)]^{4+}$ in 0.1 mol dm^{-3} NH_3 aqueous buffers at pH 9.2: (*a*) $^{14}NH_3$, (*b*) $^{15}NH_3$

a small amount of $CoP(O)(O_2)$ could be detected at 77 K. After thawing and purging with Ar overnight, comparatively little CoP(O) was regenerated. However, if the solution was heated to 80 °C for 1 h whilst purging with Ar, the CoP(O)-type spectrum was reproduced with most of its original intensity. This indicates that the main product of prolonged oxygenation is the μ-peroxy dimer. The non-quantitative recovery of the original metalloporphyrin can be attributed to the formation of some $[Co^{III}(tmpyp)]^{5+}$, which is the product obtained when an aqueous solution of H_2tmpyp^{4+} and Co^{2+} are refluxed in air overnight.[12]

$[Co^{II}(tmpyp)]^{4+}$ *in the Presence of* N *Ligands.*—The e.s.r. spectrum of a frozen solution of $[Co^{II}(tmpyp)]^{4+}$ in water containing 0.1 mol dm^{-3} NH_3 buffer (pH 9.2) is shown in Figure 2(*a*). The spectrum is very different from that observed in pure water or succinate buffer. The first two low-field $g_{\|}$ components are resolved into triplets, showing the formation of a mono-ammine complex, CoP(N). Since the triplet splitting was much more poorly resolved than with organic soluble Co^{II} porphyrins in the presence of N ligands,[1-3] the experiment was repeated with $^{15}NH_3$. As expected (^{14}N, $I = 1$; ^{15}N, $I = \frac{1}{2}$), the two low-field components show doublet splitting [Figure 2(*b*)]. The ratio of the A_N values for ^{15}N:^{14}N is 1.5, which agrees within experimental error with that predicted from the respective magnetogyric ratios (1.40). Increasing the free $^{14}NH_3$ concentration from 0.05 to 0.5 mol dm^{-3} did not change the spectrum, showing that a bis-ammine complex is not formed under these conditions.

Very similar spectra were obtained with a wide variety of N ligands, and the calculated e.s.r. parameters are given in Table 1. Some qualitative information concerning the ease of formation of the adducts CoP(N) could be derived from the concentration of the ligand required to produce a CoP(N)-type spectrum. The trends observed could be rationalized in terms of both electronic effects (nature of ligand, pK of conjugate acid), and steric factors. Thus, with ethanolamine, 0.07 mol dm^{-3} ligand was sufficient to produce a CoP(N)-type spectrum. With diethanolamine, 0.20 mol dm^{-3} ligand gave mainly a CoP(N) spectrum, although a small CoP(O) component was still observed at a ligand concentration of 1.4 mol dm^{-3}. For triethanolamine at 4.8 mol dm^{-3}, a significant CoP(O) component was present. Triethylamine (0.14 mol dm^{-3}) gave a combination of CoP(O) and CoP(N) type spectra, whereas for the much less sterically hindered quinuclidine, 0.05 mol dm^{-3} ligand was sufficient to produce a pure CoP(N) spectrum. The e.s.r. spectrum of the quinuclidine adduct showed an inflexion in the $g_{\perp}$ peak. Similarly, Walker[1] noted that the 1:1 quinuclidine–5,10,15,20-tetra(*p*-methoxyphenyl)porphyrinatocobalt(II) {$[Co^{II}(tmpp)]$} complex differed from other adducts with this porphyrin in that the $g_{\perp}$ peak was split into two. Pyridine and 2-methylpyridine gave CoP(N)-type spectra, but with collidine (2,4,6-trimethylpyridine) a CoP(O) spectrum was observed. This apparent lack of co-ordination by collidine can be ascribed to steric factors. Similar behaviour was observed with collidine and [Co(tmpp)] in toluene glass.[2] One noteworthy ligand was pipes buffer at pH 6.8, which produced a typical CoP(N) spectrum with ^{14}N hyperfine splittings, showing that the buffer co-ordinates to the Co through one of the N atoms. This spectrum is not the result of impurities present in the pipes, since it was observed when the pipes: metalloporphyrin ratio was as low as 4:1. The buffer pipes is one of the 'biological' buffers introduced by Good *et al.*,[13] and one characteristic is its reluctance to co-ordinate with metal ions.[13] The present work shows that this characteristic cannot always be relied upon, although conditions in frozen aqueous media are rather different from those in liquid water. In contrast, with tris(hydroxymethyl)aminomethane buffer at pH 8.3, and morpholine-*N*-ethane-2-sulphonic acid buffer at pH 6.2, CoP(O)-type spectra were obtained. Imidazole at a concentration of 0.05 mol dm^{-3} produced a typical CoP(N) spectrum, but at higher

Table 1. The e.s.r. parameters for $[Co^{II}(tmpyp)]^{4+}$ with various ligands at 77 K

Ligand	$g_\perp$ [a]	$A_\perp$ [b]	$g_\parallel$ [a]	$A_\parallel$ [b]	A_N [b]	Reactivity towards O_2 at room temperature [c]
H_2O	2.443	5.4	2.036	9.0		See text
$^{14}NH_3$	2.305		2.034	7.6	1.4	Rapid and irreversible reaction to give e.s.r. silent species
$^{15}NH_3$	2.292		2.030	7.3	2.1	
NH_2Bu^n	2.289		2.039	7.1	1.3	Rapid and irreversible reaction to give e.s.r. silent species
$H_3\overset{+}{N}CH_2COO^-$ [d]	2.294		2.038	7.0	1.3	25% reversible
$H_2NCH_2CH_2OH$	2.295		2.038	7.1	1.3	Dioxygen adduct just detected. 15% reversible
$(HOCH_2CH_2)_2NH$	2.310		2.028	7.7	1.7	Dioxygen adduct observed. 50% reversible
NEt_3	2.305		2.037	7.4	1.5	Dioxygen adduct slowly formed
Quinuclidine	2.315		2.039	7.2	1.0	Dioxygen adduct initially formed, little left after 10 min. 100% reversible
Imidazole	2.307		2.054	6.7	1.7	Very reactive, e.s.r. signal lost after a few seconds. Largely irreversible
Benzimidazole	2.303		2.032	7.2	1.4	
Pyridine	2.307		2.037	7.3	1.3	Rapid reaction
4-Cyanopyridine	2.312		2.035	7.5	1.5	
4-Dimethylaminopyridine	2.306		2.044	6.9	1.1	Rapid reaction. 32% reversible
2-Methylpyridine	2.310		2.038	7.4	1.6	Dioxygen adduct observed. 30% reversible
pipes	2.306		2.033	7.6	1.5	Dioxygen adduct observed. 80% reversible
N_3^-	2.300		2.035	7.4	1.8	No dioxygen adduct detected. 100% reversible

[a] ± 0.008. [b] $\times 10^{-3}$ cm^{-1}, ± 0.5. [c] Reversibility refers to the amount of the metalloporphyrin regenerated when a solution, after prolonged oxygenation, is heated to 80 °C for 1 h in a slow stream of Ar. [d] At pH 9.8.

Table 2. The e.s.r. parameters for dioxygen adducts at 77 K

Ligand	$g_\perp$ [a]	$A_\perp$ [b]	$g_\parallel$ [a]	$A_\parallel$ [b]
H_2O	2.090	1.6	2.017	1.5
$(HOCH_2CH_2)_2NH$	2.073	1.6	2.011	0.9
NEt_3	2.078	1.6	1.991	1.2
Benzimidazole	2.071	1.5	2.011	1.0
pipes	2.070	1.5	2.015	1.0

[a] ± 0.008. [b] $\times 10^{-3}$ cm^{-1}, ± 0.5.

concentrations there were changes in the e.s.r. spectrum which indicated the partial formation of a 2:1 adduct, although no N hyperfine splitting could be resolved from this species.

Reactivity of N *Complexes with Dioxygen.*—The complexes all reacted with O_2, some very rapidly indeed, and details are given in Table 1. In a few cases, the initial formation of dioxygen adducts, $CoP(N)(O_2)$ could be detected. The e.s.r. spectra were similar to, although not identical with, that obtained in pure water, and the e.s.r. parameters are given in Table 2. In general, as might be expected, the better donor ligands increased both the reactivity of the metalloporphyrin towards O_2 to give $CoP(N)(O_2)$, and also the reactivity of the species with CoP(N) to give the e.s.r. silent μ-peroxy dimer. Although, as mentioned above, collidine does not co-ordinate through its N atom to $[Co(tmpyp)]^{4+}$, it was found that the dioxygen adduct formed in the presence of collidine was much more stable at room temperature in the presence of oxygen than when the solvent was pure water. It is possible that the collidine associates with the metalloporphyrin to give a π-complex. This possibility was discussed by Walker[2] in connection with organic-soluble cobalt(II) porphyrins.

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