REDUCTION AND SPIN TRANSITION OF SOME MACROCYCLIC Fe(III) COMPLEXES

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The ESR and absorption spectroscopies were used to study the reduction of Fe(III) tetrasulphophthalocyanine (FeTSP) and Fe(III) chloro-deuteroporphyrin (FeCDP) by ascorbic acid and cysteine. Only the monomeric form of Fe(III)TSP gives an ESR spectrum, while the dimer is ESR quiet. The complex is low-spin, and is rapidly reduced by both substrates to Fe(II)TSP. The Fe(III)CDP complex is high-spin; on exposure to the substrates, it transforms first to the low spin form which alone is reduced by the substrates to the ferrous complex. The spin states of the complexes are correlated with their catalytic activity in the oxidation of the substrates by dioxygen.

As has been shown in several cases, oxidations of organic substrates by dioxygen are catalysed by transition metal ions in their lower oxidation states[1-3]. The higher oxidation states, stable in oxygen atmosphere, are catalytically less active. The catalytic reaction should, therefore, be preceded by reduction of the ions to catalytically active lower oxidation states.

The macrocyclic Fe(III) tetrasulphophthalocyanine (Fe(III)TSP) and Fe(III) chloro-deuteroporphyrin (deuterohemin, Fe(III)CDP) complexes catalyze the oxidation of ascorbic acid and cysteine by dioxygen, but differ greatly in the catalytic effectiveness[4].

The aim of this work was to establish by means of ESR and absorption spectra whether the reduction of ferric to ferrous complexes does indeed occur, and what is the role of the spin states of the complexes. In biological systems where the central ion of the active group of the catalyst is iron, as for example in *P*-450 cytochrome, the spin state of complex has a key, though not yet well understood, function.[5,6] Information acquired for a chemical system may be of value not only for elucidating the mechanism of homogeneously catalyzed reactions of oxygen but, in addition, it may be relevant to biological systems as well.

EXPERIMENTAL

Fe(III) TSP was prepared and purified by the usual procedures[7]. Fe(III)CDP, provided by the

Central Institute of Molecular Biology AS GDR in Berlin, was isolated from bovine blood[8]. Other chemicals were of analytical grade.

The reactions of Fe(III) complexes with the substrates were carried out in the media of 0·01 to 0·1M-HCl, 0·01—0·1M-NaOH, a borate buffer according to Clark–Lubbs (H_3BO_3, KCl, NaOH) of pH = 8, and a borate buffer according to Michaelis (H_3BO_3, NaOH) of pH = 8—12. The equilibrium between the monomeric and dimeric Fe(III)TSP was studied in the mixtures 25% dimethylformamide–water and 50% dimethylformamide–water. The concentrations of the reactants for ESR measurements were [Fe(III)TSP] = $5{\cdot}0 \cdot 10^{-3}$ mol/dm^3, [Fe(III)CDP] = = $4{\cdot}2 \cdot 10^{-3}$ mol/dm^3, [ascorbic acid] = $0{\cdot}7-5{\cdot}0 \cdot 10^{-2}$ mol/dm^3, [cysteine] = $2{\cdot}5 - 5{\cdot}0 \cdot \cdot 10^{-2}$ mol/dm^3, [sodium dithionite] = $2{\cdot}5 \cdot 10^{-2}$ mol/dm^3. The stock solution of Fe(III)CDP was made up in 0·1M-NaOH.

Absorption spectra were recorded on a Unicam SP 800 B instrument using a 0·1 mm cell, in order that the optical and ESR spectra might be obtained at the same concentration.

Preliminary measurements of ESR spectra were performed on a Varian E4 spectrometer at 113 K. Other measurements were carried out on an ERS-220 spectrometer at 77 K in X-band (ν = 9·28 GHz)) with a field modulation frequency of 100 kHz and microwave power 2 and 20 mW. Magnetic field calibration was checked with diphenylpicrylhydrazyl (DPPH), g = = 2·0037 and a Radiopan (Poland) Model MJ-110R digital teslameter. The splitting for low-spin iron(III) was calculated from the spectra (Kramer's doublet) using DPPH and Mn(II)/ZnS as reference standards. The samples were prepared in a nitrogen atmosphere directly in the quarz cells, sealed, and frozen.

RESULTS AND DISCUSSION

Fe(III) *Tetrasulphophthalocyanine*

Fe(III)TSP, as other metal tetrasulphophthalocyanines, exists in aqueous solution in monomeric, dimeric, and possibly also polymeric forms. The monomer and the dimer can be distinguished by their optical spectrum, where the monomer gives rise to an absorption band at λ = 666 nm, and the dimer at λ = 634 nm (ref.[9]). Close to the absorption band of the monomer, the absorption band of Fe(II)TSP, λ = = 670 nm occurs (ref.[10]) so that both may easily be confused.

Freshly prepared solutions of Fe(III)TSP in water give no ESR spectra at 77 K. Solutions in the DMF–water mixture show three lines spectra (Kramer's doublet), typical of low-spin ferric compounds[11] (d^5, S = 1/2); the line intensity increases with increasing DMF content. The 50% DMF solution produces two superimposed signals of the low-spin Fe(III) with g_x = 1·972, g_y = 2·107, h_z = 2·186, and g'_x = = 1·954, g'_y = 2·252, g'_z = 2·541, the latter corresponding apparently to the formation of a complex with DMF. (Fig. 1) Optical spectra were recorded in parallel with the ESR spectra at the same concentration of Fe(III)TSP. Fresh aqueous solution showed a single band at λ = 634 nm, indicating that the dimer is practically the only form present (Fig. 2, curve *a*). As the DMF concentration is increased an absorption band at λ = 666 nm characteristic of the monomer appears due to a displacement of the monomer–dimer equilibrium in favour of the monomer (Fig. 2,

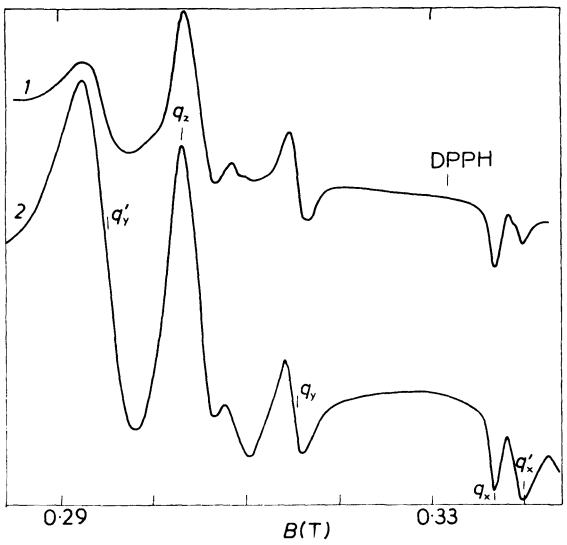

FIG. 1

ESR spectrum of Fe(III)TSP in the frozen DMF–water mixture. $5{\cdot}0 \cdot 10^{-3}$ mol/dm^3 Fe(III)TSP; N_2 atmosphere; fresh solution; $T = 77$ K; microwave power 5 mW, attenuation 18 dB, time constant 2 s, sweep rate 7·7 mT/min; *1* 25% DMF–water; *2* 50% DMF –water

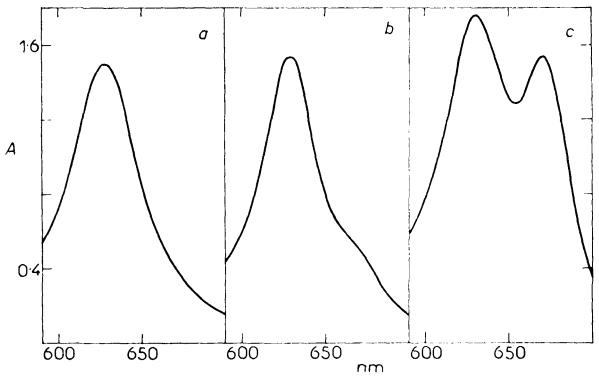

FIG. 2

Optical spectrum of Fe(III)TSP in water and in the DMF–water mixture. $5{\cdot}0 \cdot 10^{-3}$ mol/dm^3 Fe(III) TSP; 0·1 mm cell; fresh solution; *a* water; *b* 25% DMF–water; *c* 50% DMF–water

curve *b*, *c*). The appearance of the band at 666 nm is paralleled by the appearance of the ESR signal. Clearly, the ESR signal is due only to the monomeric Fe(III)TSP, the dimer being ESR quiet. After standing for several days, aqueous solutions of Fe(III)TSP produce distinct signal of the low-spin Fe(III) with $g_x = 1{\cdot}962$, $g_y = 2{\cdot}093$, and $g_z = 2{\cdot}183$. (Fig. 3).

On addition of ascorbic acid or cysteine to an acidic or alkaline medium the signal due to Fe(III) instantaneously disappears. In acid media a sharp singlet typical of a free-radical species with $g = 2{\cdot}0067$ was observed. As in the case of CoTSP (ref.[12]), it may be assigned to an intermediate of Fe(III)TSP reduction with one electron delocalized throughout the phthalocyanine ring. (Fig. 3) As ferrous compounds (d^6) do not give an ESR spectrum at the given temperature, it may be concluded that the signal disappearance is associated with the reduction of Fe(III)TSP to Fe(II)TSP. This is confirmed by the disappearance of the absorption band at 634 nm characteristic of Fe(III) species and the appearance of the band at 670 nm, assigned to Fe(II)-TSP.

Reoxidation of the reaction mixture by oxygen is not reversible, leading to just a hint of the original ESR spectrum and a new signal with $g = 4{\cdot}23$ corresponding to Fe(III) in octahedral environment with rhombic distortion. In optical spectra a rapid decrease of absorbance in the whole spectral range was observed under the

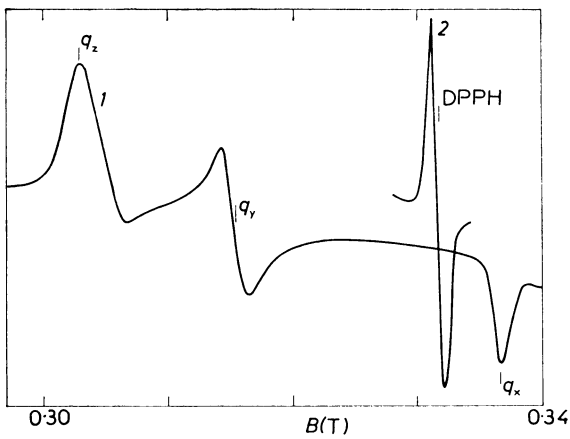

FIG. 3

ESR spectrum of Fe(III)TSP; N_2 atmosphere, $T = 77$ K, microwave power 20 mW; *1* $2{\cdot}0 \cdot 10^{-3}$ mol/dm^3 Fe(III)TSP, 20% glycerine. 19 days old solution; time constant 5 s, attenuation 30 dB, sweep rate 7·69 mT/min; *2* $5{\cdot}0 \cdot 10^{-3}$ mol/dm^3 Fe(III)TSP, $5{\cdot}0 \cdot 10^{-3}$ mol/dm^3 HCl, $7{\cdot}5 \cdot 10^{-3}$ mol/dm^3 ascorbic acid; 6 days old solution; time constant 0·2 s, attenuation 18 dB, sweep rate 14·93 mT/min

same conditions. This evidence is suggestive of oxidative degradation of the ligand. No oxidative degradation occurs in the absence of a substrate.

Fe(III) *Chlorodeuteroporphyrin*

The ESR spectrum of Fe(III)CDP in 0·1M-NaOH is characteristic for high-spin heme type Fe(III) complex with $g_\perp = 6{\cdot}08$ and $g_\parallel = 2{\cdot}00$ (d^5, $S = 5/2$) (ref.[11]). On addition of cysteine or ascorbic acid in 5–10 fold excess the intensity of the high-spin (HS) signal slightly decreases, and the spectrum shows an additional low-spin (LS) signal of Fe(III), also of the heme type[11] (Fig. 4). The intensity of the LS signal diminishes when the reaction mixture is allowed to stand with ascorbic acid, and also with increasing ascorbic acid concentration. An increase in the cysteine concentration results in disappearance of both the HS and LS signals. In the absence of substrate, the LS signal does not appear either on addition of the corresponding amount of HCl or on lowering the pH of the solution to pH = 8·72. Hence, the HS to LS transition is due to an interaction with the substrate and not to a possible change in pH caused by substrate acidity. The values of the g-factor are summarized in Table I.

After standing for several weeks, a Fe(III)CDP solution in 0·1M-NaOH showed both the HS and LS signals in its ESR spectra.

Under conditions where HS and LS signals were present in the ESR spectrum of Fe(III)CDP, interactions with substrate were followed at defined pH (Michaelis borate buffer). At pH = 8·08, both signals disappear on addition of cysteine, while

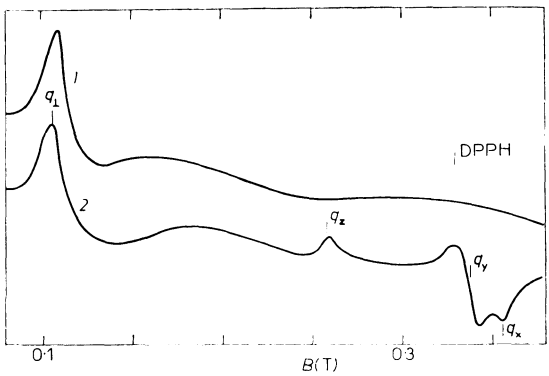

FIG. 4

ESP spectrum of Fe(III)CDP. $4{\cdot}2 \cdot 10^{-3}$ mol/dm^3 Fe(III)CDP; N_2 atmosphere; $T = 77$ K; microwave power 20 mW, attenuation 30 dB, time constant 0·1 s, sweep rate 74·63 mT/min. *1* in 0·1M-NaOH; *2* in 0·1M-NaOH, $2{\cdot}5 \cdot 10^{-2}$ mol/dm^3 ascorbic acid

no changes in the spectrum are observed on adding ascorbic acid. At pH = 12·24, cysteine again brings about marked attenuation of both signals, while ascorbic acid leads to the disappearance of the LS signal only. Reoxidation of the reaction mixture by oxygen does not restore the original spectrum, but gives rise to a signal of Fe(III) with $g \approx 4$. The addition of dithionite results in the disappearance of the LS signal. The measured values of the g-factor are given in Table I. The values for weak signals (on reduction by substrate) are approximate.

The experiments have shown that on addition of the reducing substrates, the low--spin Fe(III)TSP is instantaneously reduced to Fe(II)TSP. The high-spin Fe(III)CDP, on the other hand, is partially transformed into the low-spin form of the same complex, as evidenced by the heme character of the LS spectrum. The spin transition is presumably caused by the entry of the substrate into the coordination sphere, and is unaffected by a change in pH within the range 8 – 12.

The preferential disappearance of the LS signal after addition of ascorbic acid or dithionite under conditions where both the HS and LS signals are initially present, indicates that the LS form is reducible and the reduction occurs after the spin transition. This also implies that the reduction following the substitution and spin transition is slow, for if it were rapid, the LS signal would not be detectable. The persistence of the HS signal in the spectrum indicates that the equilibrium between the two forms of the Fe(III)CDP complex is immobile and controlled by a preceding slow reaction. In the case of cystein, whose addition causes first the appearance of LS signal and at higher concentrations the disappearance of both the HS and LS signals, the rate controlling reaction, probably the substitution, is more rapid. The LS signal is distinct in NaOH medium where the reduction of Fe(III) species by cysteine is slow[4].

The results of this study have demonstrated that both the Fe(III) complexes are reduced by substrate to Fe(II). The reduction of the low-spin Fe(III)TSP complex is rapid, consistent with the fact that FeTSP is an effective catalyst of the oxidation

TABLE I

g-Factors of the ESR Spectra of Fe(III) chloro-deuteroporphyrin

Reaction system	$g_\perp$	$g_\parallel$	g_x	g_y	g_z
0·1M-NaOH	6·08	2·00	—	—	—
pH 8 (Clark–Lubbs)	6·10	2·00	—	—	—
pH 8 (Michaelis)	6·06	2·00	1·849	1·952	2·552
pH 12 (Michaelis)	6·10	2·00	1·849	1·952	2·552
0·1M-NaOH + ascorbic acid	6·10	2·00	1·849	1·952	2·552
0·1M-NaOH + cysteine	6·10	2·00	1·97	2·32	2·44

of substrates by dioxygen. The high-spin FeCDP complex first undergoes a transition to the reducible low-spin form; the FeCDP complex acts as a poorly effective catalyst in comparison with FeTSP.

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