Comparative Study of the Optical and Magnetic Circular Dichroism Spectra of Bis-thioether and -imidazole Complexes of Iron(III) Tetraphenyl- and Octaethyl-porphyrin. Models of Haem Co-ordination in Bacterioferritins†

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The absorption and low-temperature (4.2 K) magnetic circular dichroism (MCD) spectra of the lowspin iron(III) complexes $[Fe(oep)(tht)_2]^+$, $[Fe(tpp)(tht)_2]^+$, $[Fe(oep)(Him)_2]^+$ and $[Fe(tpp)(Him)_2]^+$, where oep is 2,3,7,8,12,13,17,18-octaethylporphyrinate, tpp is 5,10,15,20-tetraphenylporphyrinate, tht is tetrahydrothiophene and Him is imidazole, have been compared to provide spectra of well defined bis-co-ordinated iron(III) porphyrin models for the diagnosis of axial haem ligation in proteins. Comparison of the shapes of the porphyrin-to-Fe^{III} charge-transfer (c.t.) bands and the magnitudes of the MCD C terms in the near-infrared region show that the electronic spectra of low-spin iron(III) complexes of oep, and hence of protoporphyrin IX (3,7,12,17-tetramethyl-8,13-divinylporphyrin-2,18dipropanoic acid), have only one transition, namely, $a_{1u}(\pi) \longrightarrow e_{g}(d)$ whereas the spectra of complexes of tpp contain two one-electron transitions a_{1u} , $a_{2u}(\pi) \longrightarrow e_g(d)$. Substitution of a phenyl group for a hydrogen atom at the porphyrin methine bridge causes a large energy shift of the $\rightarrow e_{g}(d)$ transition but leaves the other one unaffected. On changing the axial ligation of $[Fe(oep)]^+$ from bis(imidazole) to bis(tht) the energy of the $a_{1u}(\pi) \longrightarrow e_g(d)$ c.t. band shifts from 6330 (1580) to 4700 cm⁻¹ (2130 nm). The energy shift is twice that observed when bis(imidazole) ligation is changed to imidazole-thioether ligation showing that the shifts of the c.t. bands obey an approximate additivity rule. The EPR and MCD spectra of the complex [Fe(oep)(tht)₂]⁺ match well the spectra of the haem group in the bacterial iron storage protein, bacterioferritin, and hence provide strong support for the assignment of bis(methionine) co-ordination of the haem in this protein, a ligation scheme not previously reported for haem in any protein.

Many electron-transfer reactions in biology are carried out by cytochromes containing a low-spin iron porphyrin ligated by a pair of axial groups from side-chains of the polypeptide.¹ One advantage of employing low-spin iron-(II) and -(III) ions is to minimize the structural rearrangement at the metal centre on change of redox state and so to facilitate rapid electron-transfer processes. The amino acid side-chains which are capable of generating sufficiently strong ligand fields to bring about the low-spin states of Fe^{II} and Fe^{III} are histidine, cysteine, methionine and lysine.² Hence there is a total of ten possible different biaxial ligation pairs which might be utilized for electron transfer in biology. So far only four different ligation pairs have been discovered in naturally occurring proteins. X-Ray crystallography has identified bis(histidine) co-ordination in, for example, cytochrome b_5^3 and cytochrome $c_3^{4,5}$ and methionine-histidine co-ordination in cytochrome c from numerous sources⁶ and in cytochrome b₅₆₂ from *Escherichia* coli.7 Lysine-histidine co-ordination, found in cytochrome f from plants, has been established by a combination of methods including electron paramagnetic resonance (EPR) and nearinfrared (NIR) magnetic circular dichroism (MCD) spectroscopies together with evidence from protein sequence data.8 The fourth ligation pattern, discovered most recently, is bis-(methionine) co-ordination of protohaem IX [(3,7,12,17-

tetramethyl-8,13-divinylporphyrin-2,18-dipropanoato)iron] in bacterioferritin (bacfer) from Pseudomonas aeruginosa, Azotobacter vinelandii and Escherichia coli.9 This was achieved by making a comparison of the energies of the charge-transfer (c.t.) bands in the NIR MCD spectra of the low-spin iron(III) state bacfer with those of a model bis(thioether) complex, namely (2,3,7,8,12,13,17,18-octaethylporphyrinato)bis(tetrahydrothiophene)iron(III), [Fe(oep)(tht)₂]⁺. This paper reports a characterization and assignment of the absorption, MCD and EPR spectra of this and related model compounds. In the course of this work a comparison has been made between the spectroscopic properties of $[Fe(oep)(tht)_2]^+$ and $[Fe(tpp)(tht)_2]^+$ where tpp is 5,10,15,20-tetraphenylporphyrinate. The latter porphyrin ring system has been rather commonly used as a haem model system.¹⁰ We have discovered that the c.t. spectra of low-spin iron(III) complexes of the tpp ring are different in form and total intensity from those of oep and protoporphyrin IX (3,7,12,17-tetramethyl-8,13-divinylporphyrin-2,18-dipropanoic acid) rings. These differences between the spectra of the two ring systems persist for both bis(imidazole) and bis(thioether) complexes.

The optical spectra of low-spin iron(III) porphyrin complexes with bis(axial) ligation contain electronic transitions at wavelengths between 800 and 3000 nm which are assigned to c.t. transitions from the highest occupied molecular orbitals (HOMOs) of the porphyrin π system (a_{1u}, a_{2u}) to the hole in the t_{2g}^{5} d shell of Fe^{III.2} These transitions can be most readily located by MCD spectroscopy. Although the wavelength region beyond ≈ 1400 nm is partially obscured by the vibrational

[†] Submitted in celebration of the 150th anniversary of the Royal Society of Chemistry at the invitation of the Dalton Editorial Board. Non-SI unit employed: $G = 10^{-4}$ T.

Table 1EPR g values of bis-co-ordinated complexes of iron(III)porphyrins and bacterioferritins

Complex	g_z	g_y	g_x	Δ/λ^a	V/λ^b	V/Δ^{c}
$[Fe(oep)(Him)_2]^+$	2.97	2.24	1.49	3.35	1.80	0.54
$[Fe(tpp)(Him)_2]^+$	2.89	2.29	1.58	3.35	2.05	0.61
$[Fe(oep)(tht)_2]^+$	2.94	2.30	1.43	2.75	1.80	0.65
$[Fe(tpp)(tht)_2]^+$	2.89	2.37	1.46	2.49	1.93	0.78
Bacterioferritins						
Ps. aeruginosa	2.86	2.32	1.48	2.71	1.97	0.73
E. coli	2.88	2.31	1.46	2.72	1.91	0.70
A. vinelandii	2.88	2.31	1.46	2.72	1.91	0.70
R. sphaeroides	2.87	2.32	1.48	2.72	1.95	0.72

^{*a*} The energy separation, in units of iron spin-orbit coupling (λ) , between the orbitals d_{xy} and the midpoint of d_{xz} and d_{yz} . ^{*b*} The energy separation, in units of iron spin-orbit coupling (λ) , between the d_{xz} and d_{yz} orbitals. ^{*c*} Formulae in ref. 19 were used.

overtones of the protein and the water solvent the MCD intensity of vibrational transitions is at least three orders of magnitude less than that of the electronic transitions.¹¹ Hence the MCD spectrum contains features arising only from the c.t. bands. The energy of the c.t. transition depends upon the energy of the t_{2g} orbitals of Fe^{III} and hence upon the nature of the axial ligands to the metal ion. It has been possible to construct an empirical scale of the energies of the c.t. bands for given axial ligation types which may be used in combination with EPR spectroscopy to assign the nature of the axial ligation of haem groups in proteins.²

Although the designation of axial ligation type can now be made with some confidence the complete assignment of the electronic states involved has not been achieved. The two lowest-energy c.t. transitions expected arise from the oneelectron excitations, a1u- $\rightarrow e_{g}(\bar{d})$ and $a_{2u} \rightarrow e_g(d)$, where a_{1u} and a_{2u} are the porphyrin π HOMOs and e_g are the $d_{xz,yz}$ orbitals of the iron(III) t_{2g} sub-shell.^{12,13} Both transitions are predicted to be polarized in the porphyrin plane and to give MCD C terms of positive sign.¹³ These expectations have been confirmed by polarized, single-crystal spectroscopy 14,15 and by low-temperature MCD studies.² However, these studies do not establish whether only one or both of the transitions lie in the NIR. The differences between the c.t. spectra of $[Fe(oep)L_2]^+$ and $[Fe(tpp)L_2]^+$ answer this question and lead to a clear assignment of these transitions. Hence the methodology is put on a sound theoretical basis.

Materials and Methods

The complexes [Fe(tpp)Cl] and [Fe(oep)Cl] were purchased from Aldrich. Imidazole was recrystallized from ethanol and sublimed. Tetrahydrothiophene (tht) was vacuum distilled immediately prior to use. Solvents were dried by standard methods. Water-sensitive iron(III) complexes were handled in an inert-atmosphere ($O_2 < 1$ ppm) glove-box or in Schlenk tubes.

The complex $[Fe(oep)(tht)_2]ClO_4$ was prepared by dissolving $[Fe(oep)(OClO_3)]^{16}$ (12 mg) in tht (0.3 cm³), followed by addition of CHCl₃ (1 cm³). The reaction mixture was gently refluxed for 5–15 min, filtered and allowed to cool. Dichloromethane (2 cm³) was added. The bis(tht) complex of $[Fe(tpp)(B_{11}CH_{12})]^{17,18}$ was prepared in a similar way. Solutions of bis(imidazole) complexes of [Fe(oep)Cl] and [Fe(tpp)Cl] were prepared in CH₂Cl₂–CHCl₃ (2:1 v/v) by addition of excess of ligand until no further change was observed in the absorption spectrum.

The mixed organic solvents $CH_2Cl_2-Et_2O$ (2:1 v/v) and $CH_2Cl_2-CHCl_3$ (2:1 v/v) from glasses of good optical quality at low temperature suitable for MCD measurements. The glass

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volume contracts on cooling from 300 to 4.2 K by a factor of 2/3. Depolarization of the circularly polarized light beam was less than 5%. Addition of the second organic solvent, either CHCl₃ or Et₂O, to solutions of the models in CH₂Cl₂ did not alter the absorption spectrum other than by the expected dilution factor.

EPR spectra were recorded with an ER-200D X-band spectrometer (Bruker Spectrospin) interfaced to an ESP 1600 computer and fitted with a liquid-helium flow cryostat (ESR-9, Oxford Instruments). Magnetic circular dichroism spectra were measured using a split-coil superconducting solenoid, type SM-4 (Oxford Instruments), capable of a maximum field of 5 T, and either a circular dichrograph, JASCO J-500D, for the wavelength range 300–1000 nm, or a laboratory-built dichrograph² for the range 800–3000 nm. In order to extend measurements from 2000 to 3000 nm the monochromator (0.6 m, Spex Industries) was fitted with a grating blazed at 3 μ m (300 lines per mm) and a blocking filter for order sorting.

Spin quantitations were carried out against standard 1 mmol dm⁻³ (ethylenediamine-N,N,N',N'-tetraacetate)cuprate(II) in aqueous solution.

Results

A comparison has been made between the absorption, EPR and MCD spectra of the four complexes $[Fe(oep)L_2]^+$ and $[Fe(tpp)L_2]^+$ where L = imidazole (Him) or tht. Tetrahydrothiophene was introduced by Reed and co-workers^{17,18} as a model of the thioether side-chain of a methionine residue since this ligand is less volatile than Me₂S. Bis co-ordination of iron(III) porphyrin by thioether ligands is weak. Co-ordinating anions such as Cl⁻ are not readily displaced in organic solvents. Therefore, it is necessary to use iron(III) porphyrins with weakly co-ordinating anions such as ClO₄⁻, SbF₆⁻ or, most usefully, the carborane anion $[B_{11}CH_{12}]^{-17,18}$

Fig. 1 shows the absorption spectrum in CH_2Cl_2 of [Fe(oep)(OClO₃)] and [Fe(tpp)(B₁₁CH₁₂)] after the addition of a 100-fold molar excess of tht under anaerobic conditions. The bis complex is fully formed as judged by the lack of spectral change on addition of further ligand. The bis(imidazole) complexes were generated in a similar manner, either from the carborane or the chloride complex since in this case the added ligand co-ordinates sufficiently strongly to displace any anion.

The absorption spectra of $[Fe(tpp)(Him)_2]^+$, $[Fe(oep)-(Him)_2]^+$ and $[Fe(tpp)(tht)_2]^+$ show the presence of a single, low-spin iron(III) complex. On the other hand the spectrum of $[Fe(oep)(tht)_2]^+$ is clearly a mixture of species, probably lowand high- (or admixed intermediate) spin forms. The broad Soret band between 350 and 420 nm suggests the presence of more than one species. The pronounced peak at ≈ 640 nm may be indicative of a high-spin form. This was confirmed by an examination of the MCD spectrum of the sample at room temperature between 300 and 2400 nm. Bands of shape characteristic of high-spin haem were observed at 640 nm and between 800 and 1600 nm.

EPR Spectra.-The EPR spectra, recorded at 10 K, of the four bis complexes in CH₂Cl₂ are compared in Fig. 2. In each case the main feature of the spectrum is a set of rhombic g values characteristic of low-spin iron(III) porphyrin, Table 1. Spin integrations show that in every case over 95% of the sample is contributing to this low-spin signal. The derivative signals at g = 5.84 and 5.78 in the spectra of [Fe(tpp)(tht)₂]⁺ and [Fe(oep)(tht)₂]⁺ can be assigned either to a high-spin or an admixed intermediate-spin species. However, the spin integrations show these to be a minor species (<5%). This implies that the low-spin complex $[Fe(oep)(tht)_2]^+$ has become almost the exclusive species >95% low spin at 10 K even though it is clearly a mixture of low- and high- or intermediatespin species at room temperature. The sample used for the lowtemperature EPR measurement was the same as that examined at room temperature by MCD spectroscopy. It appears that the



Fig. 1 Absorption spectra of (a) $[Fe(tpp)(Him)_2][B_{11}CH_{12}]$ in $CH_2Cl_2-Et_2O$ (2:1 v/v), (b) $[Fe(tpp)(tht)_2][B_{11}CH_{12}]$ in $CH_2Cl_2-CHCl_3$ (2:1 v/v), (c) $[Fe(oep)(Him)_2]ClO_4$ in $CH_2Cl_2-Et_2O$ (2:1 v/v) and (d) $[Fe(oep)(tht)_2]ClO_4$ in $CH_2Cl_2-CHCl_3$ (2:1 v/v)



Fig. 2 EPR spectra of bis complexes of low-spin iron(11) porphyrins. Compounds as in Fig. 1. Temperature 10 K, power 2.02 mW, frequency 9.39 GHz, modulation amplitude 10 G, solvents as in Fig. 1

binding constant of tht for [Fe(oep)]⁺ increases significantly on lowering the temperature. This is consistent with expectations based on entropic considerations. The g values compare well with those reported by Reed and co-workers¹⁷ for the tht complexes and with those reported earlier²⁰ for the bis(imidazole complexes), Table 1.

The low-symmetry distortion parameters of the crystal field acting at Fe^{III} can be determined from the g value using the

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Fig. 3 Visible-region MCD spectra of bis complexes of low-spin iron(III) porphyrins. Compounds as in Fig. 1. Temperature 4.2 K, magnetic field 5 T, solvents as in Fig. 1



Fig. 4 NIR region MCD spectra of bis complexes of low-spin iron(III) porphyrins. Compounds as in Fig. 1, conditions as in Fig. 3

formulae of Taylor.¹⁹ The two parameters are the axial distortion Δ , corresponding to the energy separation between the d_{xy} and the baricentre of the d_{xz}, d_{yz} orbitals, and V, the rhombic distortion, equal to the energy gap ($d_{yz} - d_{xz}$). The table also contains the g values observed for the haem group in bacfer samples isolated from four different bacterial organisms. The g values and the low-symmetry crystal-field parameters of

bacfer are remarkably similar to one another and surprisingly invariant to the source. This suggests a close structural similarity between them. The parameters of the EPR spectra of the low-spin bis(tht) complexes are distinct from those of the bis(imidazole) complexes. The axial distortion of the tht complex, Δ/λ of 2.49–2.75, is considerably lower than that of the bis(imidazole) complex, Δ/λ of 3.35. Given that the thioether is



Fig. 5 Absorption spectra of (a) $[Fe(oep)(Him)_2]ClO_4$ (30 mmol dm³ in $CH_2Cl_2-Et_2O$) and (b) $[Fe(tpp)(Him)_2][B_{11}CH_{12}]$ (4 mmol dm³ in $CH_2Cl_2-CHCl_3$)

a weaker field ligand than imidazole the axial distortion is expected to be lower. Table 1 shows that the complex which most closely matches that of the haem group in bacfer on the basis of the EPR parameter alone is $[Fe(oep)(tht)_2]^+$. This similarity extends to the MCD spectra as well (see later).

MCD Spectra.—The MCD spectra of the four model complexes in CH_2Cl_2 — $Et_2O(2:1 v/v)$ have been recorded at 4.2 K and 5 T over the wavelength range 300–2800 nm. They are compared in Figs. 3 and 4 in two regions, 300–800 and 800–2800 nm. The features appearing in the latter region are discussed first.

Near infrared. Fig. 4 enables a comparison to be made between the effect of changing the axial ligation type from bis(tht) to bis(imidazole) and of changing the porphyrin ring system from oep to tpp. The spectra arise from the porphyrinto-Fe^{III} c.t. transitions. Hence, changes in axial ligation and in the porphyrin structure lead to alterations in the spectra. A change in the axial ligation state from bis(imidazole) to bis(tht) gives rise, in both porphyrin ring systems, to a shift in the energy of the band to the red by $\approx 1650 \text{ cm}^{-1}$. For example, the peak of the c.t. band of [Fe(oep)L₂]⁺ moves from 1580 to 2130 nm as L is changed from imidazole to tht. Although the band maxima are somewhat less well defined, it is clear that a similar overall shift is observed for the tpp ring system.

The second feature is that the form of the spectrum changes when the porphyrin ring system is altered from oep to tpp. The c.t. spectra, when tpp is the axial ring system, appear to contain extra intensity in the form of a new band on the high-energy side of the band system observed with oep. In order to confirm that these extra features persist in absorption the spectra of the bis(imidazole) complexes of $[Fe(oep)]^+$ and $[Fe(tpp)]^+$ were measured in the NIR at room temperature, Fig. 5. Care was taken to balance out the overtone absorption from the sample and reference beams. It proved impossible to obtain comparable absorption spectra of the tht derivatives because the c.t. bands lie at long wavelengths and are difficult to disentangle from the vibrational overtone absorption. The spectra, Fig. 5, show that similar band shapes are found at room temperature. The bands are not as well resolved; neither are the band widths as sharp as in the low-temperature MCD spectra. This is undoubtedly due to the different temperatures at which the samples were measured. In the following section the MCD and absorption spectra are analysed by the moments method in order to establish the number of electronic transitions which underlie these bands and to compare the magnitudes of the *C* terms with those predicted by theory.²¹

Analysis. The MCD spectra of low-spin iron(III) haems at 4.2 K are dominated by the contributions from the C term, which varies inversely as the absolute temperature.²² The spectra of the four complexes studied here are temperature dependent (data not shown). The MCD magnetization curves show that the species dominating the spectra arise from the low-spin iron(III) complexes. At 5 T, the field at which the spectra were recorded, and 4.2 K the MCD spectra are no longer linearly dependent upon the applied magnetic field, since partial magnetic saturation occurs. However, the intensities of the spectra can be corrected, provided that the ground-state g values are known, to give values which would be observed at 4.2 K and 5 T if the paramagnets were obeying the Curie law, that is the approximation of the linear limit can be used.

In the absence of a contribution from B or A terms the zeroth moment of the MCD spectrum can be expressed as in equation (1) and the zeroth moment of the absorption spectrum as in (2),

$$\left(\frac{\Delta \varepsilon}{v}\right)_0 = 326.6 \frac{C_0}{kT} \beta B \tag{1}$$

$$(\varepsilon/v)_0 = 326.6D_0 \tag{2}$$

where β is the Bohr magneton, *B* the applied magnetic field, *k* Boltzmann's constant and *T* the absolute temperature. The zeroth moment of a spectroscopic parameter, α , which varies as a function of energy, v, is defined as in equation (3) where the

$$(\alpha)_0 = \int \alpha(\nu) d\nu \tag{3}$$

integration is between the points where $\alpha(v)$ falls to zero on either side of a peak or trough. The c.t. bands of Fig. 4 are hence ideally suited for a moments analysis since they are isolated from other bands.

Table 2 gives the values of the zeroth moments of the MCD spectra at 4.2 K and 5 T obtained by summation over the complete band envelopes shown in Fig. 4. The zeroth moments of the absorption spectra measured at room temperature can only be determined in the case of two of the complexes, from the data in Fig. 5. In the spectrum, of the tpp derivative, Fig. 5(b), there is partial overlap with the visible region of the absorption spectrum. Hence an extrapolation has been made to enable a moment to be calculated.

The results in Table 2 show that the total C-term intensities as measured by the zeroth moment of the MCD of the bis complexes of $[Fe(tpp)]^+$ are approximately double those of $[Fe(oep)]^+$. In the case of the bis(imidazole) complexes the ratio is 1.80:1 whereas for the bis(tht) complexes it is 2.17:1. For the dipole strengths of the bis(imidazole) complexes, determined from the zeroth moment of the absorption spectrum, the ratio is 2.20:1. These results show clearly that the c.t. spectra of the tpp ring system contain approximately twice as much intensity as those of the oep ring. This suggests that two c.t. transitions are present in the spectra of the tpp complexes compared with only one in the latter case. Further evidence in support of this can be obtained by examination of the absolute magnitudes of the C terms and by comparison with the values predicted by theory.

The one-electron c.t. transitions, $a_{1u}(\pi) \longrightarrow e_g(d)$ and $a_{2u}(\pi) \longrightarrow e_g(d)$, are predicted to show positive C terms in the MCD spectrum. The magnitudes of the C term for each transition can be calculated [see equation (7), ref. 21] and are

Table 2	Moments analysis of the	NIR MCD spectra of bis-co-c	ordinated complexes of	low-spin iron(III) porphyrins
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Complex	$\int (\Delta \epsilon^* / \nu) d\nu^a / dm^3 \text{ mol}^{-1} \text{ cm}^{-1}$	$\int (\epsilon/v)/dv^{b} dm^{3} mol^{-1} cm^{-1}$	C_0 / D_0^{c}	$g_z a b / (a^2 + b^2)^d$
[Fe(tpp)(Him) ₂] ⁺	79.5	164.2	0.87	0.69
$[Fe(oep)(Him)_2]^+$	44.1	74.6	1.04	0.78
$[Fe(tpp)(tht)_2]^+$	108.5	n.d.	n.d.	0.73
$[Fe(oep)(tht)_2]^+$	49.9	n.d.	n.d.	0.77

^{*a*} Zeroth moment of MCD spectrum, $\int (\Delta \epsilon/\nu) d\nu$, between the wavelength limits 800 and 2800 nm. ^{*b*} Zeroth moment of absorption spectrum, $\int (\epsilon/\nu) d\nu$, between the wavelength limits 800 and 2000 nm. ^{*c*} $C_0/D_0 = (\Delta \epsilon/\epsilon)(kT/\beta B)K$ where k is Boltzmann's constant, T is the absolute temperature (4.2 K), β the Bohr magneton, B the magnetic field (5 T) and K is a term to correct the magnitude of C_0 to be linear in field at 4.2 K. ^{*d*} g_z is obtained from the experimental EPR spectrum, see Table 1; a and b are the coefficients of the d_{xz} and d_{yz} orbitals in the ground-state Kramers doublet, calculated from the values of Δ and V, Table 1, according to the formulae of Taylor.¹⁹

predicted to be the same. The ratio C_0/D_0 depends only upon the parameters of the electronic ground state as in equation (4)

$$C_0/D_0 = g_z ab/(a^2 + b^2)$$
(4)

where a and b are the coefficients of the d_{yz} and d_{xz} orbitals in the ground state. They are uniquely determined by the three components of the ground-state g tensor, as shown by Taylor.¹⁹ Thus $a = (g_z + g_y)/4K$, $b = (g_z - g_x)/4K$ and 4K = 2.83 $(g_z + g_y - g_x)^{\frac{1}{2}}$. Hence the value of C_0/D_0 is uniquely determined by the three components of the ground-state g value. Table 2 shows the values determined for the four complexes from the experimental g values given in Table 1. An experimental value of C_0/D_0 can be determined only in the case of the bis(imidazole) complexes since the D_0 values are available only in these instances. Agreement between the observed and predicted values of C_0/D_0 is obtained for both ring systems, namely 0.87 and 0.69 and 1.04 and 0.78, respectively, for tpp and oep, Table 2. This agreement is satisfactory taking into account the different temperatures at which the MCD and absorption spectra were measured.

In the case of two overlapping transitions, which is postulated to be the case for $[Fe(tpp)L_2]^+$, it can be shown that the zeroth moment over the complete MCD band is proportional to the sum of the C terms of the individual transitions and the zeroth moment over the absorption band is likewise proportional to the sum of the dipole strengths of each transition [ref. 23, equations (7.6.1) and (7.6.6)]. Hence the zeroth moments of the MCD and absorption bands are expected to be double in the case of the tpp ring system compared with oep. However, the ratio of the two moments is expected to remain invariant to the ring type.

This agreement provides strong support for the assignment of the NIR c.t. bands. The fact that the *C*-term intensity and the dipole strength of the band system in $[Fe(tpp)]^+$ complexes is about double that in similar complexes of $[Fe(oep)]^+$ implies that in the former case two one-electron transitions, a_{1u} and $a_{2u} \longrightarrow e_g(d)$, are present whereas in the latter case only one transition is present. The shape of the band envelopes supports this conclusion.

The MCD spectra alone do not provide a criterion for deciding which one of the transitions is present in the spectra of the complexes of $[Fe(oep)]^+$. However, a plausible assignment can be made by inspection of the orbital coefficients of the a_{1u} and a_{2u} orbitals. This aspect is considered in the Discussion section.

Visible and Soret region. Fig. 4 shows the MCD spectra at 4.2 K and 5 T of the four complexes under study. They are similar to those of bis(histidine), bis(imidazole) and methionine-histidine co-ordinated protohaem IX in the same spectral regions.²⁴ These regions are therefore not usefully diagnostic of axial ligation. The assignment of the additional bands to long wavelength of the α band is unclear. A number of low-spin iron(III) haemoproteins including metmyoglobin azide and horse-heart cytochrome c show optical absorption bands in this region which are polarized perpendicular to the haem plane.¹⁵

Several assignments have been invoked including c.t. from axial ligand to Fe^{III} , or porphyrin $a_{2u}(\pi)$ to $\text{Fe}^{\text{III}} d_{z^2}$. Fig. 4 shows that a change of the porphyrin ring system shifts the energies of these bands. This argues against their assignment as axial ligand to Fe^{III} c.t. The band at 690 nm in the spectrum of [Fe(oep)(tht)₂]⁺ is of particular interest. Since the work described in the previous section makes clear that in the case of [Fe(oep)]⁺ complexes only one of the two transitions $a_{1u} \longrightarrow e_g$ lies in the NIR it is possible that the band at 690 nm represents the second of these two transitions. The $\Delta \varepsilon$ value of the peak is $\approx 200 \text{ dm}^3 \text{ mol cm}^{-1}$ at 4.2 K and 5 T which is the same intensity as that of the band in the near infrared. It would be helpful to know the linear polarization of this band to see whether or not it has appreciable z polarization. The band can be located in the absorption spectrum of bacfer and is also seen in the MCD spectra of the latter. There is also a band at a similar wavelength in the spectrum of cytochrome c, the so-called 695 nm band. It is known that the energy of this band is sensitive to the state of axial ligation of cytochrome c. If the methionine ligand is displaced from the haem group the band is lost from the spectrum.

Discussion

The spectroscopic studies presented in this work aim to establish the validity of using bis-co-ordinated complexes of low-spin iron(III) porphyrins as suitable models for the iron(III) protoporphyrin IX ring in naturally occurring proteins. The first comparison drawn has been between complexes of oep and tpp ring systems. The complex $[Fe(tpp)]^+$ has been commonly used to model haemoprotein chemistry because of the ease of synthesis and structural characterization of its derivatives. It has been less successful in modelling spectroscopic features. This work shows that for optical and MCD studies $[Fe(tpp)]^+$ does not model the excited electronic states of iron(III) protoporphyrin IX well. The $[Fe(oep)]^+$ complexes are a much better match. The reasons for this are discussed below.

The second comparison has been between low-spin iron(III) porphyrin complexes with two co-ordinated imidazole ligands and those with two thioether ligands. The properties of proteins containing protohaem IX co-ordinated by two histidine residues are well studied. However, the co-ordination by two thioether groups as in bis(methionine) ligation has only been discovered recently in bacterioferritin.⁹ The purpose of this work has been to establish the similarity of the model and the protein.

NIR c.t. Spectra of $[Fe(tpp)L_2]^+$ and $[Fe(oep)L_2]^+$.—By means of a quantitative analysis of the low-temperature MCD spectra we have shown that the NIR electronic spectra of lowspin $[Fe(oep)]^+$ complexes, and iron(III) protoporphyrin IX, contain one c.t. transition whereas the spectra of low-spin $[Fe(tpp)]^+$ complexes contain both transitions $a_{1u}(\pi) \longrightarrow e_g(d)$ and $a_{2u}(\pi) \longrightarrow e_g(d)$. The MCD spectra alone do not provide a criterion for deciding which of the two transitions is present in the former case. However, a plausible assignment can be made by inspection of the orbital coefficients of the a_{1u} and a_{2u} HOMOs at the methine bridge carbon atoms of the porphyrin ring, Fig. 6. The magnitude and signs of the coefficients obtained from the four-orbital model of Gouterman and Wagniere²⁵ are



Fig. 6 The HOMOs and LUMOs of the porphyrin ring. The magnitudes of the molecular orbital coefficients are indicated by the size of the circles centred on each carbon atom. The sign of the coefficient is denoted by dotted or continuous lines; ± 4 and ± 5 given in units of $h/2\pi$ are z components of the orbital angular momentum of the free-electron orbitals corresponding to the HOMOs and LUMOs shown.

shown as circles. The a_{1u} orbital has nodal planes passing through the methine bridges giving zero electron density at these carbon atoms. Substitution of the methine bridge hydrogen atoms by phenyl groups, as in the change from oep to tpp, will leave the energy of this orbital unaffected in a firstorder approximation. Thus the energy of the $a_{1u}(\pi) \longrightarrow e_g(d)$ c.t. transition should be relatively unaffected by substituents at the methine bridges. Therefore we assign the coincident transition present in $[Fe(oep)]^+$ and $[Fe(tpp)]^+$ to the a_{1u} - $\rightarrow e_{a}(d)$ orbitals. Conversely, the energy of the a_{2u} orbital is expected to be markedly affected by phenyl substitution at the methine bridge. Therefore we propose that the higher-energy transition, $a_{2n}(\pi)$ $\rightarrow e_g(d)$ which is present in the c.t. spectrum of $[Fe(tpp)]^+$, has been shifted either to higher or lower energy out of the NIR region for [Fe(oep)]⁺. There is a possibility that the positive peak at 690 nm in the MCD spectrum of $[Fe(oep)(tht)_2]^+$, Fig. 3, is this transition, $a_{2u}(\pi)$ $e_{g}(d)$.

The forms of the NIR c.t. spectra of the complexes of $[Fe(tpp)]^+$ with imidazole and tht are rather different. It is clear from the quantitative analysis of the MCD spectra that two oneelectron c.t. transitions lie under the band envelope. The excited states arising from the one-electron configurations $(a_{1u})^1(e_g)^4$ and $(a_{2u})^1(e_g)^4$ are ${}^2A_{1u}$ and ${}^2A_{2u}$, respectively. These states are not mixed by interelectronic repulsion since the orbital symmetries are different. However, spin-orbit coupling can mix them via the component L_zS_z because L_z transforms as A_{2g} under D_{4h} . Second-order spin-orbit coupling between the excited states can cause intensity redistribution between them. The overall total intensity will not, however, be changed. Hence the moments analysis will not reveal this interaction.

We can conclude that the c.t. band observed in the NIR region of low-spin iron(III) complexes of oep and protoporphyrin IX consists of only a single one-electron transition and that is a $a_{1u}(\pi) \longrightarrow e_g(d)$. This is contrary to many of the assumptions made in previous discussions of this band system.^{13,26} The shape of the band must arise from the presence of vibrational side-bands stretching to higher energy. The rather prominent



Fig. 7 Comparison between the MCD spectra of (a) $[Fe(oep)(tht)_2]^+$ and (b) bacterioferritin (*Pseudomonas aeruginosa*). Spectra were measured at 4.2 K and 5 T

peak to higher energy has been assigned as the second electronic transition.²⁶ This is incorrect. The energy gap between the main peak and this band remains remarkably constant over a series of complexes between 1400 and 1500 cm⁻¹. This falls within the range of typical porphyrin skeletal-mode frequencies of totally symmetric vibrations which are expected to couple to the porphyrin electronic states.

 $E_{c.t.}$ of Bis(tht) and Bis(imidazole) Complexes of [Fe(oep)]⁺. -The energy of the $a_{1u}(\pi) \longrightarrow e_g(d)$ c.t. band shifts from 6330 (1580) to 4700 cm^{-1} (2130 nm) on changing the axial ligation from bis(imidazole) to bis(tht). It has been shown² in a range of proteins that the c.t. band for bis(histidine) co-ordination lies between 6250 and 6750 cm⁻¹ and for histidine-methionine coordination lies between 5250 and 5750 cm⁻¹. Taking average values this corresponds to a shift of ≈ 950 cm⁻¹. Results on a wide variety of proteins and models show that the energy of the c.t. band, $E_{c.t.}$, can be predicted by means of an additivity rule. Each ligand type can be assigned an energy parameter such that addition of this parameter for each ligand yields $E_{\rm c.t.}$. The parameters are: methionine, 2250; histidine, 3200; lysine, 3750; histidinate, 4100; and cysteine, 6000 cm⁻¹. The predicted value of $E_{c.t.}$ for a bis(methionine) [or bis(thioether)] complex is 4500 cm⁻¹ in close agreement with the value of 4650 cm⁻¹ obtained for the model bis(tht) complex. Thus this additivity relationship apparently holds over a wide range of ligand-field strengths and can be used with some confidence to predict the values of $E_{c,t}$ of hitherto unknown bis(axial) ligation states.

Comparison of the MCD spectra of bacterioferritin (Pseudomonas aeruginosa) with [Fe(oep)(tht)₂]⁺.—The model complex which most closely resembles that of the haem site in bacterioferritin is [Fe(oep)(tht)₂]⁺. In order to permit a comparison to be made Fig. 7 shows the MCD spectra of 4.2 K and 5 T from 300 to 2600 nm of this model complex and bacterioferritin from *Pseudomonas aeruginosa*.²⁷ The EPR g values are given in Table 1. There are two spectral regions of the MCD spectra which are most distinctive, namely between 600 and 750 and between 1000 and 2500 nm. The region between 300 and 600 nm is typical of low-spin iron(III) haem coordinated by a pair of ligands and is not significantly changed as the ligation state of the haem group is varied amongst the four models under consideration. The match between the pair of spectra is not perfect in the other two regions. However, no other combinations of axial ligands give peaks at these two wavelengths. Some variation in peak wavelength and peak intensity can be caused in proteins by structural variations at the haem site. These may include, for example, the relative orientations of the axial ligand planes to one another, the iron(III)-ligand distance permitted by protein side-chain constraints and the polarity of the haem environment. Since the transitions being discussed are c.t., relatively high transition dipoles will be created and hence the polarity of the surroundings can cause shifts due to relaxation. Given these factors the match between the spectra of the model compound and the protein provides strong evidence for bis(methionine) coordination being present in bacterioferritin. The striking similarity between the EPR g values and the NIR MCD spectra

of bacterioferritin proteins from four different bacterial sources implies that the haem binding site is structurally very similar in all these proteins.

Acknowledgements

This work has been supported by the SERC under the Molecular Recognition Initiative and by National Institutes of Health grant GM-23851. We thank Dr. G. R. Moore, Dr. F. Kadir, Professor C. Greenwood and Professor P. M. Harrison for supplying samples of proteins and for helpful discussions.

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Received 4th January 1991; Paper 1/00057H