[October, 1982] © 1982 *The Chemical Society of Japan* *Bull. Chem. Soc. Jpn.*, *55*, 3059—3063 (*1982*)

Origins and Spin Dependence of Near Infrared Magnetic Circular Dichroism of Iron(III) Hemoproteins

Takao YAMAMOTO,† Tsunenori NOZAWA, Nagao KOBAYASHI,†† and Masahiro HATANO*
Chemical Research Institute of Non-aqueous Solutions, Tohoku University, Sendai 980

(Received October 5, 1981)

Temperature variation experiments on near infrared magnetic circular dichroism spectra (700—2000 nm) have demonstrated that metmyoglobin (iron(III) myoglobin) cyanide and metmyoglobin fluoride derivatives which are typical low and high spin iron(III) heme complexes, respectively, exhibit the Faraday *C* term and the Faraday *C* and *A* terms, respectively. The results are consistent with theoretical predictions based on the assignment that the near infrared bands of the iron(III) heme complexes are attributable to the charge-transfer transitions from the occupied a_1 and a_2 π orbitals to the vacant iron $d\pi$ (d_{xz}, d_{yz}) orbitals in a C_{4v} notation.

The magnetic circular dichroism (MCD) spectra of heme for the ultraviolet and visible bands which consist mainly of porphyrin π to π^* transitions have reflected the electronic states of the heme iron.[1)] The origins of the MCD have been explained in several ways.[1–10)] Beyond the visible region, hemoproteins show several transitions ascribable to d-d transitions or to charge-transfer transitions associated with the d-electrons in the near infrared region,[11,12)] so that these MCD bands should give more direct information of spin, oxidation, and ligand states of hemoproteins.

Cheng *et al.*[13)] measured near infrared MCD spectra of methemoglobin (iron(III) hemoglobin) cyanide in the wavelength region from 800 to 2000 nm. Later, Stephens *et al.*[14)] extended their studies to methemoglobin derivatives with fluoride, water, and azide. They explained all the MCD spectra as being composed of Faraday *C* terms in the charge-transfer transitions. Near infrared MCD spectra of various derivatives of metmyoglobin and myoglobin (iron(II) myoglobin) have been reported by Nozawa *et al.*[15)] Near infrared MCD studies on cytochrome c′,[16)] peroxidase,[17)] P-450,[18)] and cytochrome c[19)] have appeared recently.

In this paper, assignment of Faraday parameters has been established experimentally by temperature variation studies on the typical low spin (metmyoglobin cyanide) and high spin (metmyoglobin fluoride) iron(III) hemoproteins. Further, the results are discussed in comparison with theoretical calculations.

Experimental

Horse heart myoglobin used was purchased from Sigma (type III). All chemicals were guaranteed grade reagents and used without further purification. ^2H_2O phosphate buffer (1/15 mol dm^{-3}, pH 6.98) and H_2O phosphate buffer (0.01 mol dm^{-3}, pH 7.0) were used to dissolve metmyoglobin cyanide and metmyoglobin fluoride, respectively. Potassium hexacyanoferrate(III) was used to oxidized myoglobin completely and excess hexacyanoferrate(III) was removed by passage through a column of Sephadex-G25 (medium). Metmyoglobin derivatives were prepared by adding respective solid salts to appropriate concentration (1 mol dm^{-3} KCN, 1.5 mol dm^{-3} NaF). Undissolved fraction was removed by low speed centrifugation (600 g). Amicon ultra filters (CF-25) were used to concentrate metmyoglobin solutions by the centrifugation.

Absorption spectra were recorded on a Hitachi EPS-3T spectrophotometer. MCD spectra from 800 to 1000 nm were obtained by use of a JASCO Model J-20A CD spectrometer with S-20 and S-1 type photomultipliers (Hamamatsu Television). The sensitivity of the J-20A is as high as 1×10^{-3} degree cm^{-1}. MCD spectra beyond 1000 nm were measured on a JASCO Model J-200 CD spectrometer with a CaF_2-photoelastic modulator (Morvue) incorporated. An S-1 type photomultiplier (Hamamatsu Television) and an InSb photovoltaic cell (Judson) were used as detectors for the wavelength regions from 800 to 1100 and beyond 1000 up to 2000 nm, respectively. The J-200 CD instrument has sensitivities of 2×10^{-3} and 5×10^{-3} degree cm^{-1} for the photomultiplier (800—1100 nm) and photovoltaic cell regions (1000—2000 nm), respectively. Electromagnets capable of producing magnetic fields up to 1.48 and 1.14 T were used for MCD measurements. The MCD magnitude is expressed by the molar ellipticity per Tesla ($[\theta]_M/10^4$ degree mol^{-1} dm^3 m^{-1} T^{-1} or 10 degree m^2 mol^{-1} T^{-1}).

Measurements at room temperature were carried out in 1/15 mol dm^{-3} ^2H_2O potassium phosphate buffer (pH 6.98). For low temperature measurements, mixtures of phosphate buffer and ethylene glycol-d_6 (50 : 50 in volume) and of phosphate buffer, potassium glycerophosphate, glycerol, and hydrochloric acid solution (4 : 20 : 10 : 5 in volume) were used in the regions from 1000 to 2000 (for metmyoglobin cyanide) and from 700 to 1000 nm (for metmyoglobin fluoride), respectively, to get transparent glass down to the liquid nitrogen temperature; the temperature was monitored with an alumel-chromel thermocouple inserted in the cell placed in a quartz Dewar vessel, and controlled by a stream of cold gaseous nitrogen evaporated from liquid nitrogen. Each scan was started 30 min after the temperature had reached a constant value. MCD measurements were repeated at least three times, results of a set being in good agreement with one another whithin experimental errors (±5%).

Results

Figure 1 demonstrates the effect of temperature on near infrared MCD and absorption spectra of metmyoglobin cyanide which is a typical low spin derivative. The MCD and absorption spectra at 0 °C show a good agreement with those previously reported by us.[15)] The inset in Fig. 1 shows that the $[\theta]_M$ values of the MCD peaks at 6390 cm^{-1} (1560 nm), 7810 cm^{-1} (1280 nm),

---

[†] Present address: Kawamura Research Institute, 2-7-8, Kamikizaki, Urawa, Saitama 338.

[††] Present address: Pharmaceutical Institute, Tohoku University, Aobayama, Sendai 980.

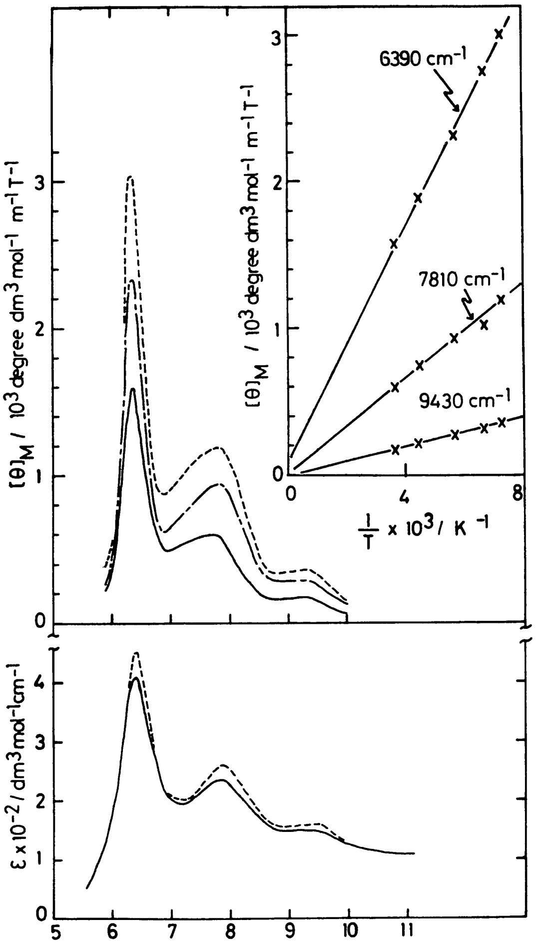

Fig. 1. Near infrared MCD and absorption spectra for metmyoglobin cyanide in the near infrared region at various temperatures(——; 273 K, — - —; 176 K, -----; 137 K). In the inset, the $[\theta]_M$ values are plotted against reciprocals of absolute temperatures. Metmyoglobin concentration, 3.83×10^{-3} mol dm^{-3} (on the heme basis), KCN concentration, 1×10^{-3} mol dm^{-3}, 1.48 T. Cell length, 3 mm. Other experimental conditions are described in the text.

and 9430 cm^{-1} (1060 nm) increased in proportion to the reciprocal of absolute temperature ($1/T$). The extraporation of the lines gives zero intercepts at $1/T=0$. The absorption spectra exhibit only small increases in magnitude and band narrowing. These facts clearly indicate that the near infrared MCD spectra of metmyoglobin cyanide are composed of pure Faraday C terms. The intervals of the three peaks are 1420 and 1620 cm^{-1} and the intensity ratio among them is 93 : 35 : 1 at 0 °C.

Figure 2 illustrates the MCD spectra of metmyoglobin fluoride, a typical high spin iron(III) heme complex, at 271, 219, 179, and 150 K. Figure 3 shows the corresponding absorption spectra. The MCD and absorption spectra at 273 K agree with those previously reported by us.[15] The MCD peak around 12100 cm^{-1} (830 nm) exhibited only a small increase in intensity and a red shift by 400 cm^{-1} with temperature change from 271 and 150 K. The trough around 10600 cm^{-1} (940 nm) showed a red shift of 500 cm^{-1} and a 179% intensity increase. The absorption spectra also exhibited a 500 cm^{-1} red shift and an increase in magnitude around 11000 cm^{-1} by 80%. These complicated changes in the absorption and MCD spectra indicate that the near infrared MCD spectra are composed of a few Faraday

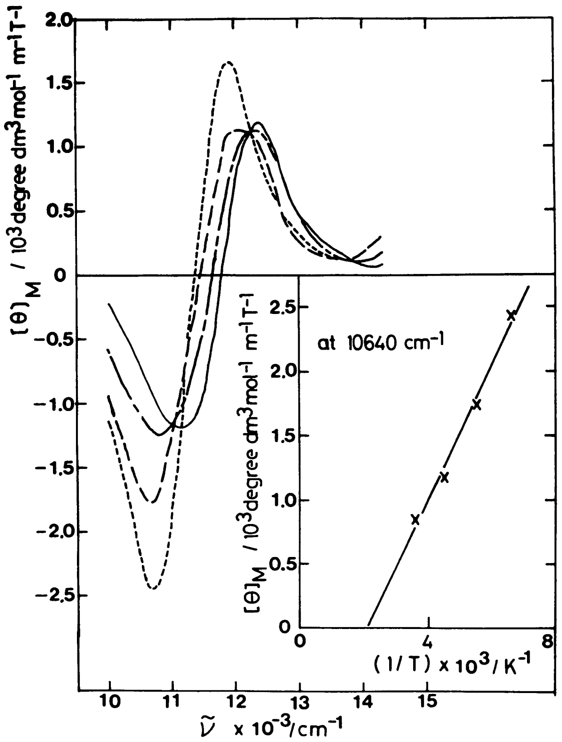

Fig. 2. MCD spectra for metmyoglobin fluoride in the near infrared region at various temperatures (——; 271 K, — - —; 219 K, ----; 179 K, and -----; 150 K). Metmyoglobin concentration, 6.04×10^{-3} mol dm^{-3}, NaF concentration, 1.5 mol dm^{-3}, cell length 2 mm, magnetic field 1.14 T. Other experimental conditions are described in the text.

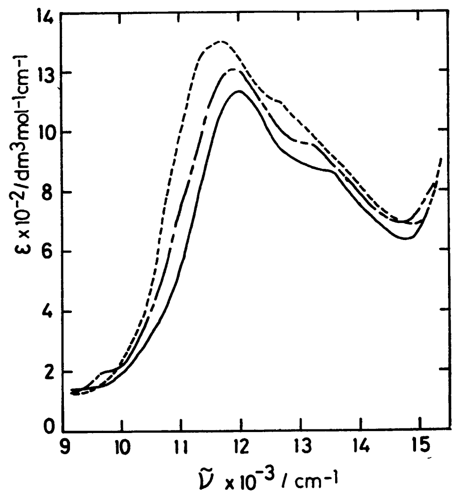

Fig. 3. Absorption spectra for metmyoglobin fluoride in the near infrared region at several temperatures (——; 293 K, — - —; 213 K, -----; 143 K). Other experimental conditions are the same as those for Fig. 2.

terms. The large temperature dependence of the MCD spectra suggests the presence of Faraday C term (around 10600 cm^{-1}). The temperature-independent Faraday A term with the S-shaped dispersion is also presumed to be present around 11500 cm^{-1}. Since the absorption spectra also increased in magnitude and exhibited a red shift, some changes in the MCD spectra should be due to that in the absorption spectra. Therefore it is concluded that the near infrared MCD spectra are composed of the large A term[20] around 11500 cm^{-1} and the C term around 10600 cm^{-1}.

Discussion

Electronic States of Heme. Actual heme chromophore has no C$_{4v}$ symmetry rigorously. However, many studies have shown that it is a good approximation in the first order.[21] In this approximation the 3d iron has five orbitals with four different energies conventionally designated as b$_2$ (d$_{xy}$ or ζ), e (d$_{xz}$ or η, d$_{yz}$ or ξ), a$_1$ (d$_z$^{2} or u), and b$_1$ (d$_{x^2-y^2}$ or v) as shown in Fig. 4. The evidence from experiments and theories showed that the near infrared band in the Fe(III) hemoproteins should be the charge-transfer transition from the porphyrin highest occupied π orbitals (a$_1$ and a$_2$) to the iron vacant d-orbitals (e) in a C$_{4v}$ approximation.[11,12]

Iron(III) Low Spin. Since a lower symmetry perturbation lifts the degeneracy in the e orbitals and the spin-orbit interaction mixes the t$_{2g}$ (O$_h$) orbitals, the ground electronic states of the low spin iron(III) heme can be represented as the following Kramers' doublet:[22–25]

$$\begin{aligned}\phi_k{}^+ &= -A_k\phi_\xi{}^+ + \mathrm{i}B_k\phi_\eta{}^+ + C_k\phi_\zeta{}^-,\\ \phi_k{}^- &= A_k\phi_\xi{}^- + \mathrm{i}B_k\phi_\eta{}^- + C_k\phi_{\zeta^+},\end{aligned}\tag{1}$$

where

$$\begin{aligned}\phi_\xi{}^+ &= |\mathrm{a}_1\bar{\mathrm{a}}_1\mathrm{a}_2\bar{\mathrm{a}}_2\xi\eta\bar{\eta}\zeta\bar{\zeta}|, & \phi_\xi{}^- &= |\mathrm{a}_1\bar{\mathrm{a}}_1\mathrm{a}_2\bar{\mathrm{a}}_2\bar{\xi}\eta\bar{\eta}\zeta\bar{\zeta}|,\\ \phi_\eta{}^+ &= |\mathrm{a}_1\bar{\mathrm{a}}_1\mathrm{a}_2\bar{\mathrm{a}}_2\xi\bar{\xi}\eta\zeta\bar{\zeta}|, & \phi_\eta{}^- &= |\mathrm{a}_1\bar{\mathrm{a}}_1\mathrm{a}_2\bar{\mathrm{a}}_2\xi\bar{\xi}\bar{\eta}\zeta\bar{\zeta}|,\\ \phi_\zeta{}^+ &= |\mathrm{a}_1\bar{\mathrm{a}}_1\mathrm{a}_2\bar{\mathrm{a}}_2\xi\bar{\xi}\eta\bar{\eta}\zeta|, & \phi_\zeta{}^- &= |\mathrm{a}_1\bar{\mathrm{a}}_1\mathrm{a}_2\bar{\mathrm{a}}_2\xi\bar{\xi}\eta\bar{\eta}\bar{\zeta}|.\end{aligned}\tag{2}$$

In the Slater determinant, bar implies down spin and no bar stands for up spin. Similarly the charge-transfer state from the porphyrin a$_1$ (π) orbital to metal ξ, η (d$_\pi$) can be described as

$$\begin{aligned}\phi_4{}^+ &= -\ |\mathrm{a}_1\mathrm{a}_2\bar{\mathrm{a}}_2\xi\bar{\xi}\eta\bar{\eta}\zeta\bar{\zeta}|,\\ \phi_4{}^- &= -\ |\bar{\mathrm{a}}_1\mathrm{a}_2\bar{\mathrm{a}}_2\xi\bar{\xi}\eta\bar{\eta}\zeta\bar{\zeta}|.\end{aligned}\tag{3}$$

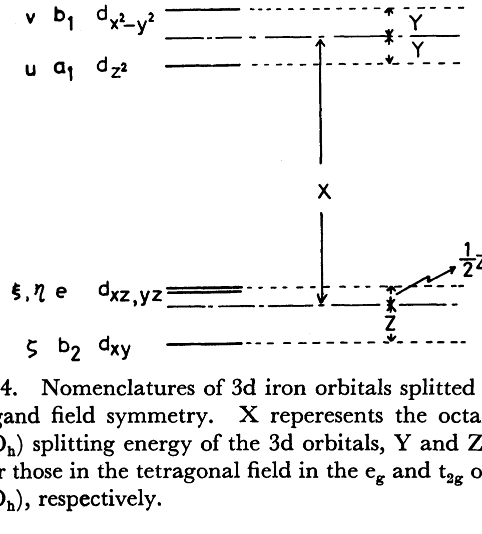

Fig. 4. Nomenclatures of 3d iron orbitals splitted in C$_{4v}$ ligand field symmetry. X reperesents the octahedral (O$_h$) splitting energy of the 3d orbitals, Y and Z stand for those in the tetragonal field in the e$_g$ and t$_{2g}$ orbitals (O$_h$), respectively.

Table 1. A set of coefficients and relative energies for respective Kramers' doublet

k	Relative energy	A_k	B_k	C_k
1	0	0.877	0.439	−0.188
2	1.417 λ[a]	0.463	−0.881	0.100
3	3.656 λ[a]	0.122	0.175	0.977

a) λ represents a spin-orbit coupling coefficient.

Electron paramagnetic resonance experiments for mct-myoglobin cyanide give three g values as follows:[26]

$$g_x = 0.75,\ g_y = 1.88,\ g_z = 3.41.$$

Since these g values should arise from the Zeeman levels at the lowest Kramers' doublet, the relations expressed by

$$\begin{aligned}g_x &= 2|(B_1-C_1)^2 - A_1{}^2|,\\ g_y &= 2|(A_1-C_1)^2 - B_1{}^2|,\\ g_z &= 2|(A_1+B_1)^2 - C_1{}^2|,\end{aligned}\tag{4}$$

together with a normalization condition ($A_k{}^2+B_k{}^2+C_k{}^2=1$) give the A_k, B_k, and C_k values listed in Table 1, which also contains coefficients and relative energies for the second and third Kramers' doublets derived from a method similar to that of Griffith[23] and Kotani.[24]

Iron(III) High Spin. The ground state of the iron(III) high spin heme belongs to the ^{6}A state described as follows for the state with $M=5/2$:

$$\phi_6 = |\mathrm{a}_1\bar{\mathrm{a}}_1\mathrm{a}_2\bar{\mathrm{a}}_2\xi\eta\zeta\mathrm{uv}|,\tag{5}$$

and the other five wavefunctions for the ^{6}A state can be obtained by operating the shift operator $\mathbf{S}_-$ on Eq. 5. In high spin derivatives, the lowest charge-transfer transitions are from a$_1$, a$_2$, to b$_2$, e. These give rise to a

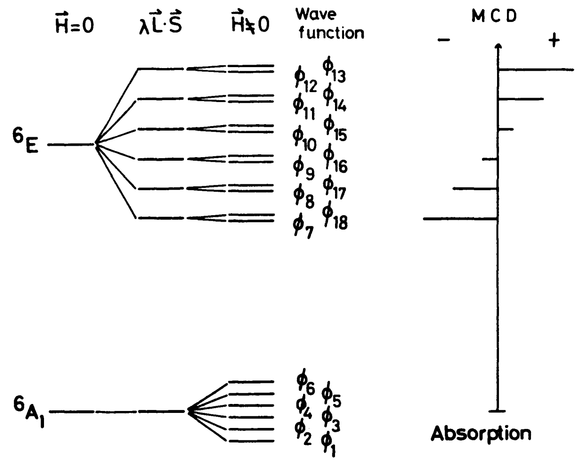

Fig. 5. The splitting pattern for the ground (^{6}A$_1$) and excited states (^{6}E) of the charge-transfer band in the high spin iron(III) heme derivatives by the effect of the spin-orbit coupling $\lambda\cdot\vec{L}\cdot\vec{S}$ and the magnetic field (H). The sign and relative magnitudes of Faraday C terms for the respective sublevels are also illustrated in the figure.

variety of states. However, to the first order in spin-orbit coupling only the states to which electric dipole transitions are allowed are the two ^{6}E states arising from e←a_1 and e←a_2, whose wavefunctions diagonalized in μ_z and $\lambda\cdot\vec{L}\cdot\vec{S}$ can be expressed for $M=5/2$ as follows:

$$\phi_{18} = -\frac{i}{\sqrt{2}}\left\{\left|\eta\frac{5}{2}\right> + i\left|\xi\frac{5}{2}\right>\right\},$$

$$\phi_{12} = -\frac{i}{\sqrt{2}}\left\{\left|\eta\frac{5}{2}\right> - i\left|\xi\frac{5}{2}\right>\right\}. \tag{6}$$

Similarly, the other diagonalized wavefunctions can be obtained as linear combinations of the two components with $M=-5/2$, $3/2$, $-3/2$, $1/2$, and $-1/2$. A schematic diagram for the iron(III) high spin ground and charge-transfer excited states are shown in Fig. 5 in the absence and presence of applied magnetic field.

Prediction of Faraday Parameters and Comparison with Experimental Results. Now we see how the MCD theory and the electronic states of the heme predict the kind and values of Faraday parameters for the charge-transfer transitions in the Fe(III) low and high spin heme complexes, and will compare calculated results with experimental data.

To calculate the Faraday parameters the following formulas[2–4] are used:

$$[\theta]_M = -24NH_z/\hbar c[f_1A + f_2(B+C/kT)], \tag{7}$$

$$A = (3/d_a)\sum_{a\to j}[\langle j|\mu_z|j\rangle - \langle a|\mu_z|a\rangle]\times \mathrm{Im}\{\langle a|m_x|j\rangle\langle j|m_y|a\rangle\}, \tag{8}$$

$$B = (3/d_a)\sum_{a\to j}\mathrm{Im}\{\sum_{k\neq a}[\langle k|\mu_z|a\rangle/(E_k-E_a)]\cdot [\langle a|m_x|j\rangle\langle j|m_y|k\rangle - \langle a|m_y|j\rangle\langle j|m_x|k\rangle] + \sum_{k\neq j}[\langle j|\mu_z|k\rangle/(E_k-E_j)]\cdot[\langle a|m_x|j\rangle\langle k|m_y|a\rangle - \langle a|m_y|j\rangle\langle k|m_x|a\rangle]\}, \tag{9}$$

$$C = (3/d_a)\sum_{a\to j}\langle a|\mu_z|a\rangle\mathrm{Im}\{\langle a|m_x|j\rangle\langle j|m_y|a\rangle\}, \tag{10}$$

$$f_1 = \frac{\pi^{1/2}\nu(\nu_{ja}-\nu)}{h\,\Delta_{ja}{}^3}\exp\left[-\frac{(\nu-\nu_{ja})^2}{\Delta_{ja}{}^2}\right], \tag{11}$$

$$f_2 = \frac{\pi^{1/2}\nu}{2\Delta_{ja}{}^2}\exp\left[-\frac{(\nu-\nu_{ja})^2}{\Delta_{ja}{}^2}\right]. \tag{12}$$

The symbol μ_z represents the z component of the magnetic moment operator and m_x and m_y stand for the x and y components of the electric moment operators. The number of degeneracies in the ground state is expressed by d_a. The summations are taken over all degenerate transitions for j←a.

Iron(III) Low Spin. According to the Boltzmann distribution and the energy separations among the three Kramers' doublets in Table 1, the populations in the lowest Kramers' doublets at 288 K were calculated to be 95 and 79% for the assumed spin-orbit coupling coefficient of 400 and 200 cm^{-1}, respectively. Hence in the following calculations, the contributions from the two higher Kramers' doublets were neglected.

By using the parameters A_1, B_1, and C_1 listed in Table 1 and Eqs. 1—3 and 7—11, one gets the Faraday A, B, and C terms as follows:

$$\begin{aligned} A &= 0.81\,\langle a_1|m_x|\eta\rangle\langle\xi|m_y|a_1\rangle, \\ B &= -1.84\times10^{-3}\,\langle a_1|m_x|\eta\rangle\langle\xi|m_y|a_1\rangle, \\ C &= -1.96\,\langle a_1|m_x|\eta\rangle\langle\xi|m_y|a_1\rangle. \end{aligned} \tag{13}$$

The extrema in the MCD spectra will be given as follows by assuming a Gaussian line shape:[27]

$$[\theta]_M^{\max}(A) = (\pi^{1/2}e^{-1/2}\nu_{ja}/\sqrt{2}\,h\Delta_{ja}{}^2)A, \tag{14}$$

$$[\theta]_M^{\max}(B+C/kT) = (\pi^{1/2}\nu_{ja}/2\Delta_{ja})(B+C/kT). \tag{15}$$

Here ν_{ja} is the frequency of transition and Δ_{ja} is the band width at $[\theta]_M=(1/e)[\theta]_M^{\max}$. The ratios of the maximum values of the Faraday parameters given in Eqs. 14 and 15 can be estimated as follows:

$$[\theta]_M^{\max}: \frac{C/kT}{B} = \frac{1}{kT}\cdot\frac{(-1.96)}{(-1.84\times10^{-3})} = 5.19, \tag{16}$$

$$[\theta]_M^{\max}: \frac{C/kT}{A} = \frac{\sqrt{e}\,h\Delta_{ja}(-1.96)}{\sqrt{2}\,kT\;\;(0.81)} = -6.88, \tag{17}$$

where we adopted $\Delta_{ja}=500$ cm^{-1} and $kT=205$ cm^{-1}. These results predict that Faraday C terms will dominate in the MCD spectra of the Fe(III) low spin complexes even at 295 K. This agrees with the experimental result that the near infrared MCD spectra of the metmyoglobin cyanide are predominantly composed of Faraday C terms.

Iron(III) High Spin. In this case the spin-orbit interaction makes the excited states split into six sublevels as shown in Fig. 5. Hence Faraday parameters should be calculated for the six transitions to these six excited state sublevels. The calculated Faraday parameters A and C are:

$$\begin{aligned} A &= (3/2)\langle a_1|m_x|\eta\rangle\langle\xi|m_y|a_1\rangle \quad \text{for all six transitions}, \\ C &= (-15/2)\langle a_1|m_x|\eta\rangle\langle\xi|m_y|a_1\rangle \\ &\quad \text{for } (\Phi_{12}, \Phi_{13}) \longleftarrow (\Phi_1, \Phi_6), \\ &\quad (-9/2)\langle a_1|m_x|\eta\rangle\langle\xi|m_y|a_1\rangle \\ &\quad \text{for } (\Phi_{11}\ \Phi_{14}) \longleftarrow (\Phi_2, \Phi_5), \\ &\quad (-3/2)\langle a_1|m_x|\eta\rangle\langle\xi|m_y|a_1\rangle \\ &\quad \text{for } (\Phi_{10}, \Phi_{15}) \longleftarrow (\Phi_3, \Phi_4) \\ &\quad (3/2)\langle a_1|m_x|\eta\rangle\langle\xi|m_y|a_1\rangle \\ &\quad \text{for } (\Phi_9, \Phi_{16}) \longleftarrow (\Phi_3, \Phi_4), \\ &\quad (9/2)\langle a_1|m_x|\eta\rangle\langle\xi|m_y|a_1\rangle \\ &\quad \text{for } (\Phi_8, \Phi_{17}) \longleftarrow (\Phi_2, \Phi_5), \\ &\quad (15/2)\langle a_1|m_x|\eta\rangle\langle\xi|m_y|a_1\rangle \\ &\quad \text{for } (\Phi_7, \Phi_{18}) \longleftarrow (\Phi_1, \Phi_6). \end{aligned} \tag{18}$$

Calculations indicate that so long as the sublevels of the ground and excited states are considered, the Faraday B term makes no contribution to the MCD spectra for this charge-transfer band. Since the signs of the six Faraday A terms are the same, the intensity of the Faraday A terms for the overall ^{6}E←^{6}A will be additive. On the other hand, the Faraday C terms for the transitions from (Φ_1, Φ_6) to (Φ_{12}, Φ_{13}) and (Φ_7, Φ_{18}) are of equal magnitude but of opposite signs, hence they partly cancel with one another. The effects of these addition and cancellations were calculated analytically by taking λ (spin-orbit coupling coefficient) to be equal to 200 cm^{-1} and $\Delta_{ja}=1000$ cm^{-1}. The calculated results indicated that the Faraday A term increased by a factor of about 6, but that the Faraday C term reduced to 1/4 of that of the largest component. Hence the ratio of the maximum intensity of the resultant Faraday terms will be

$$[\theta]_{\mathrm{M}}^{\max}: \frac{C/kT}{A} = \frac{f_2(15/2)\times(1/4)}{kTf_1(3/2)\times 6} = 1.24 \quad \text{at } T = 273\ \mathrm{K}, \qquad (19)$$
$$= 2.25 \quad \text{at } T = 150\ \mathrm{K}.$$

The values suggest that at 273 K the Faraday *A* term contribution is comparable to that of the Faraday *C* term, but that at 150 K the contribution from the Faraday *C* term becomes larger until at very low temperature the Faraday *C* term predominates in the near infrared MCD spectra. The contribution of the Faraday *C* terms extracted from the experimental results is rather small as compared with the values estimated above. Magnitudes of the estimated *C* terms are more sensitive to change in those of the band width (Δ_{ja}) and spin-orbit coupling constant (λ) than that of the *A* term, because the former is a difference and the latter is an addition of overlapping component bands. The magnitude of the estimated *C* term becomes smaller as Δ_{ja} increases and λ decreases. Although the set of parameters used in the calculation is one of reasonable sets, the difference between the estimated and experimental values may imply that Δ_{ja} is larger than 1000 cm^{-1} and that λ is smaller than 200 cm^{-1}.

In conclusion, the near infrared MCD bands for the typical low (metmyoglobin cyanide) and high (metmyoglobin fluoride) spin iron(III) heme complexes have experimentally been determined to be composed of Faraday *C* terms and Faraday *C* and *A* terms, respectively. The theoretical calculations of Faraday parameters are consistent with the experimental Faraday parameters.

The authors acknowledge the support from Grant-in-Aid for Scientific Research of the Japanese Government.

References

1) M. Hatano and T. Nozawa, "Advances in Biophysics," ed by M. Kotani, S. Yomosa, and M. Hatano, Japan Scientific Societies Press, Tokyo and University Park Press, Baltimore (1978), Vol. 11, p. 95.

2) A. D. Buckingham and P. J. Stephens, *Ann. Rev. Phys. Chem.*, **17**, 399 (1966).

3) P. N. Schatz and A. J. McCaffery, *Quart. Rev. Chem. Soc.*, **23**, 552 (1969).

4) P. J. Stephens, *Ann. Rev. Phys. Chem.*, **25**, 201 (1974).

5) A. Kaito and M. Hatano, *J. Am. Chem. Soc.*, **100**, 2034 and 4037 (1978) and references therein.

6) J. I. Treu and J. J. Hopfield, *J. Chem. Phys.*, **63**, 613 (1975).

7) M. A. Livshitz, A. M. Arutyunyan, and Yu. A. Sharonov, *J. Chem. Phys.*, **64**, 1276 (1976).

8) H. Kobayashi, T. Higuchi, and K. Eguchi, *Bull. Chem. Soc. Jpn.*, **48**, 457 (1976).

9) Y. Seno, N. Kameda, and J. Otsuka, *J. Chem. Phys.*, **72**, 6048, 6059 (1980).

10) Yu. A. Sharonov, A. P. Mineyev, M. A. Livshitz, N. A. Sharonova, V. B. Zhurkin, and Yu. P. Lysov, *Biophys. Struct. Mech.*, **4**, 139 (1978).

11) M. Zerner, M. Gouterman, and H. Kobayashi, *Theor. Chim. Acta*, **6**, 363 (1966).

12) D. W. Smith and R. J. P. Williams, *Struct. Bonding*, **7**, 1(1970).

13) J. C. Cheng, G. A. Osborne, P. J. Stephens, and W. A. Eaton, *Nature*, **241**, 193 (1973).

14) P. J. Stephens, J. C. Sutherland, J. C. Cheng, and W. A. Eaton, "Proceedings of the International Conference on Excited States of Biological Molecules, Leiden," ed by J. Birks, Wiley-Interscience, New York (1976), p. 434.

15) T. Nozawa, T. Yamamoto, and M. Hatano, *Biochim. Biophys. Acta*, **427**, 28 (1976).

16) J. Rawlings, P. J. Stephens, L. A. Nafie, and M. D. Kamen, *Biochemistry*, **16**, 1725 (1977).

17) N. Kobayashi, T. Nozawa, and M. Hatano, *Biochim. Biophys. Acta*, **493**, 340 (1977).

18) T. Nozawa, T. Shimizu, M. Hatano, H. Shimada, T. Iizuka, and Y. Ishimura, *Biochim. Biophys. Acta*, **534**, 285 (1978).

19) N. Kobayashi, T. Nozawa, and M. Hatano, *Bull. Chem. Soc. Jpn.*, **54**, 919 (1981).

20) Since heme b in myoglobin has not the true C_{4v} symmetry when all the substituents on the periphery of the porphyrin are taken into consideration, the porphyrin lowest unoccupied e orbitals (C_{4v}) are slightly split into two closely positioned orbitals in the actual symmetry lower than C_{4v}. Hence there can not be expected the true Faraday *A* term. However, as the calculation indicated (A. Kaito *et al.*, *Chem. Phys. Lett.*, **52**, 154 (1977)), the Faraday *B* terms predominantly mix among these two split orbitals to give an S-shaped MCD band which is a mimic Faraday *A* term. In this paper this apparent *A* term has been treated just like the true Faraday *A* term.

21) Y. Seno and J. Otsuka, "Advances in Biophysics," ed by M. Kotani, S. Yomosa, and M. Hatano, Japan Scientific Societies Press, Tokyo and University Park Press, Baltimore (1978), Vol. 11, p. 13.

22) M. Weissbluth, *Struct. Bonding*, **2**, 1 (1967).

23) J. S. Griffith, *Nature*, **180**, 30 (1957).

24) M. Kotani, *Suppl. Progr. Theor. Phys.*, **17**, 4 (1961).

25) H. Kobayashi, "Advances in Biophysics," ed by M. Kotani, University of Tokyo Press, Tokyo and University Park Press, Baltimore (1976), Vol. 8, p. 191.

26) G. Shulman, S. H. Glarum, and M. Karplus, *J. Mol. Biol.*, **57**, 93 (1971).

27) P. J. Stephens, W. Suetaka, and P. N. Schatz, *J. Chem. Phys.*, **44**, 4592 (1966).