BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 43 2335—2341 (1970)

Magnetic Circular Dichroism of Iron Tetraphenylporphins

Hiroshi KOBAYASHI, Masayuki SHIMIZU and Ichiro FUJITA

Department of Chemistry, Tokyo Institute of Technology Ookayama, Meguro-ku, Tokyo

(Received January 12, 1970)

The magnetic circular dichroism of iron tetraphenylporphins was measured. Since the ground state of the open-shell iron porphin is degenerate and several charge-transfer excited states exist near the ^1Q and ^1B states, the A term as well as the B and C terms are very important for describing the MCD of the iron porphin, whereas the MCD at the ^1Q and ^1B bands, which arise from the pure degenerate (π,π^*) excitations in D_{4h} porphin assumed for zinc tetraphenylporphin, is ascribed to the A term. The MCD of carboxyhemoglobin, oxyhemoglobin and also deoxyhemologin was observed and compared with that of the synthesized iron porphins.

Iron porphyrin plays an essential rôle in the biological processes of hemoprotein. Current interest leans to the studies of biological processes by means of physical measurement and theoretical calculations based on molecular physics. Magnetic optical rotatory dispersion (MORD) and magnetic circular dichroism (MCD) measurements are physical techniques recently developed to obtain information on the electronic behavior in a molecule.[1) MORD measurements of myoglobin,[2) hemoglobin[3) and cytochromes,[4) which are hemoproteins

---

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

[2) M. V. Volkenstein, J. A. Sharonov and A. K. Shemelin, *Nature*, **209**, 710 (1966).

[3) V. E. Shashoua, "Hemes and Hemoproteins," ed. by B. Chance, R. W. Estabrook and T. Yonetani, Academic Press, New York (1966), p. 93.

[4) V. E. Shashoua, *Nature*, **203**, 972 (1964); *Arch. Biochem. Biophys.*, **111**, 550 (1965); V. E. Shashoua and R. W. Estabrook, "Hemes and Hemoproteins," ed. by B. Chance, R. W. Estabrook and T. Yonetani, Academic Press, New York (1966), p. 427; M. Morrison and J. Duffield, *ibid.*, p. 431.

containing Fe(II)- or Fe(III)porphyrins, have been carried. Schooley *et al.* also reported the MCD measurements of some biologically important molecules including oxyhemoglobin, cytochrome-*c* and chlorophylls.[5]

In a magnetic field parallel to the incident light beam, the complex refractive indices of a substance for left- and right- handed circularly polarized lights, $\hat{n}_l$ and $\hat{n}_r$, respectively, are generally different because of the Zeeman splitting of the levels of orbital angular motion. The complex refractive index is given by $\hat{n}=n-ik$, where n is refractive index and k is absorption coefficient. Magnetic optical rotation arises from a magnetically induced deviation of the refractive indices for left- and right-handed circularly polarized lights, (n_l-n_r). In the region of optical absorption, a deviation in the absorption coefficients for left- and right- handed circularly polarized lights, (k_l-k_r) is observed in a magnetic field. Magnetic circular dichroism is ascribed to the difference between k_l and k_r induced by a magnetic field.

A theoretical study of the MORD observed in the region of the (π, π^*) transitions of porphyrins and phthalocyanines, first measured by Shashoua,[6] has been carried out by Stephens *et al.*[7] Since the electronic excitations corresponding to the absorption bands of iron porphyrin, however, should be interpreted in terms of a configuration-interaction admixture of the charge transfer excitations and the (π, π^*) transitions, a treatment such as employed by Stephens *et al.* can not be applied to the analysis of the MORD and the MCD of the iron porphyrins. The measurements of the MCD of the iron porphyrins have been required for further theoretical analysis of the electronic structure. In this paper, we should like to present the result of the MCD measurements of the iron tetraphenylporphins recently synthesized in this laboratory.[8]

Experimental

Materials. The free base of tetraphenylporphin (abbreviated to TPP) and Zn(II) tetraphenylporphin (TPPZn) were prepared and purified by the method given in the literature.[9] Iron tetraphenylporphins have been synthesized in this laboratory. The synthesis will be given elsewhere.[8] Samples of TPPFe(III)Cl, TPPFe(III)OCH_3, TPPFe(II)2Py, TPPFe(II)·2THF and TPPFe(II) identified by elemental analysis were used without further purification. In the formula, Py denotes the coordinating pyridine and THF the coordinating tetrahydrofuran.

Horse hemoglobin also was prepared and purified by the method in the literature.[10] Deoxyhemoglobin was prepared by adding a small amount of sodium dithionite to the buffer solution (pH 6) of freshly prepared hemoglobin. Carboxyhemoglobin was prepared by bubbling carbon monoxide into the solution of hemoglobin until the intensity of the characteristic absorption of the carboxyhemoglobin attained the highest value. The observed absorption spectra of the deoxyhemoglobin and the carboxyhemoglobin were in good agreement with those given in the literature.[10]

Preparation of the Solutions for Measurement. As solvents, tetrahydrofuran (THF) was used for TPP and TPPZn(II), benzene for TPPFe(III)Cl and TPPFe(III)OCH_3, and buffer solution (pH 6) for hemoglobins. Since ferrous tetraphenylporphins are readily autoxidized in the presence of oxygen and/or moisture in the air, the solution was prepared under inert atmosphere in a vacuum system using the solvents previously dried, distilled and degassed. The solution was transferred into a branch joined to an optical cell and the branch was then sealed off. As solvents, pyridine free from oxygen and moisture was used for TPPFe(II)·2Py, tetrahydrofuran for TPPFe(II)·2THF and benzene for TPPFe(II).

The concentration of the porphyrin in the solutions was adjusted to 10^{-4} mol/l for the measurement in the visible region and to 10^{-5} mol/l for the measurement in the near ultraviolet region.

Measurements. In a preliminary stage of this work, a permanent magnet with an 8 mm axial hole and a 13 mm pole gap was placed in the sample compartment of a JASCO automatic recording spectropolarimeter model ORD/UV-5. The external magnetic field was 3600 gauss. After a preliminary study, the light path of the spectropolarimeter was drastically modified and an electromagnet with a 15 mm pole gap was placed in the sample compartment of the spectropolarimeter. The electromagnet gave a magnetic field up to 12000 gauss. For the present work, the external magnetic field was set as 10000 gauss. When the current in the electromagnet was reversed, the reading of the out-put was completely reversed in sign over the whole range of wavenumber. A deviation between two symmetrical curves obtained for the reversed currents in the magnet, as shown in Fig. 6, gave an effective signal corresponding to one that could be obtained in a magnetic field of 20000 gauss. This technique was used for determination of the magnetic circular dichroism (MCD). The MCD of a solution was actually measured in optical density units by a deviation from the base line obtained by the first running with a pure solvent. The magnitude of MCD was given in molar ellipticity per unit external

---

[5] D. A. Schooley, E. Bunnenberg and C. Djerassi, *Proc. Natl. Acad. Sci.*, **53**, 579 (1965).

[6] V. E. Shashoua, *J. Amer. Chem. Soc.*, **86**, 2109 (1964); **87**, 4044 (1965).

[7] P. J. Stephens, W. Suëtaka and P. N. Schatz, *J. Chem. Phys.*, **44**, 4592 (1966).

[8] H. Kobayashi and Y. Yanagawa, This Bulletin, to be published.

[9] A. D. Adler, F. R. Longs, J. D. Finarelli, J. Goldmacher, J. Assour and L. Korsakoff, *J. Org. Chem.*, **32**, 476 (1967); P. Rothermund and A. R. Menotti, *J. Amer. Chem. Soc.*, **63**, 267 (1941); **70**, 1808 (1948); G. D. Dorough, J. R. Miller and F. M. Huennekens, *J. Amer. Chem. Soc.*, **73**, 267 (1951).

[10] D. L. Drabkin, "Haematin Enzymes," ed. by J. E. Falk, R. Lemberg and R. K. Morton, Pergamon Press, Oxford (1961), p. 142.

magnetic field $[\theta]_M$.[7,11] The molar ellipticity is given by the molar circular dichroism $(\varepsilon_l-\varepsilon_r)_M$ measured in a cell with 1 cm light path in a magnetic field of 10000 gauss as follows.

$$[\theta]_M = 3300\,(\varepsilon_l - \varepsilon_r)_M/10000$$

In the case of hemoglobin, even in the non-magnetic condition, it shows a circular dichroism which is ascribed to its helical structures in the counterpart of the iron porphyrin. The MCD of hemoglobin was derived from the difference between the circular dichroisms observed in the magnetic and non-magnetic conditions assuming an additivity between CD and MCD.

Results and Discussion

The structure of the coordination spheres in TPPFe(II)·2Py and TPPFe(II)·2THF is a distorted octahedron, while that of TPPFe(III)OCH_3 and TPPFe(III)Cl is a square pyramid, where the central iron might be slightly out of the porphin plane.[12,13] The structure of the coordination sphere in deoxyhemoglobin is a square pyramid[14] and that of carboxyhemoglobin is a distorted octahedron. The structure of TPPFe(II), TPP-Zn(II) and also TPP is almost planar, as shown in Fig. 1. This particular porphin has much less tendency to aggregate in solution because of the four phenyl groups perpendicular to the porphin plane.[12,15,16,] Table 1 summarizes the spin states of the iron complexes of TPP and the hemoglobins. The magnetic susceptibility measurements of the iron tetraphenylporphins have been carried out at room temperature by the Gouy method. Ferrous tetraphenylporphins were unstable in the air, so the MCD, absorption spectra and the magnetic moments were measured *in vacuo* without exposing the sample to the air.

TABLE 1.

	μ_{eff} (Bohr magneton)	S
TPP Fe(III) Cl	5.88	5/2
TPP Fe(III) OCH_3	1.84	1/2
TPP Fe(II)·2Py	0	0
TPP Fe(II)·2THF	2.75	1
TPP Fe(II)	4.75	2

	S
Carboxyhemoglobin	0
Oxyhemoglobin	0
Deoxyhemoglobin	2

The MCD and absorption spectra are shown in Figs. 2—6. Though TPPFe(II)·2THF and TPP-Fe(II) were of considerable interest from the electronic structure point of view, the observed MCD both in the visible and near ultraviolet regions was so low that no reliable value could be obtained in a magnetic field up to 10000 gauss. However, the magnitude of the MCD of TPPFe(II)·2Py was fairly large.

The magnitude of magnetic molar circular dichroism observed at a transition $a\rightarrow j$ is given[1,7,11] by

$$[\theta]_M = -\frac{24\boldsymbol{N}}{\hbar c}\{f_1A+f_2(B+C/\boldsymbol{r}T)\} \tag{1}$$

where

$$A=\frac{3}{d_a}\sum[\langle j|\hat{\mu}_z|j\rangle-\langle a|\hat{\mu}_z|a\rangle]\cdot \boldsymbol{I}_m[\langle a|\hat{m}_x|j\rangle\langle j|\hat{m}_y|a\rangle] \tag{2}$$

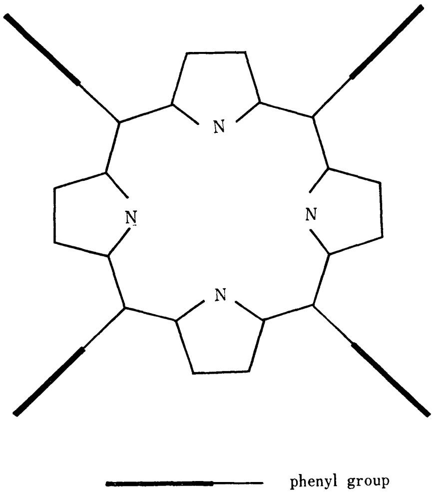

Fig. 1. Tetraphenylporphin.

---

[11] P. N. Schatz, A. J. McCaffery, W. Suëtaka, G. N. Henning, A. B. Ritchie and P. J. Stephens, *J. Chem. Phys.*, **45**, 722 (1966); P. J. Stephens, P. N. Schatz A. B. Ritchie and A. J. McCaffery, *J. Chem. Phys.*, **48**, 132 (1968); A. J. McCaffery, P. J. Stephens and P. N. Schatz, *Inorg. Chem.*, **6**, 1614 (1967); P. N. Schatz, P. J. Stephens, G. N. Henning and A. J. McCaffery, *Inorg. Chem.*, **7**, 1246 (1968); G. N. Henning, A. J. McCaffery, P. N. Schatz and P. J. Stephens, *J. Chem. Phys.*, **48**, 5656 (1968); A. J. McCaffery, P. N. Schatz and P. J. Stephens, *J. Amer. Chem. Soc.*, **90**, 5730(1968); P. J. Stephens, A. J. McCaffery and P. N. Schatz, *Inorg. Chem.*, **7**, 1923 (1968); A. J. McCaffery, P. N. Schatz and T. E. Lester, *J. Chem. Phys.*, **50**, 379 (1969).

[12] E. B. Fleischer, C. K. Miller and L. E. Webb, *J. Amer. Chem. Soc.*, **86**, 2342 (1964).

[13] J. L. Hoard, M. J. Hamor, T. A. Hamor and W. S. Caughey, *ibid.*, **87**, 2312 (1965); D. F. Koenig, *Acta Cryst.*, **18**, 663 (1965); J. L. Hoard, "Hemes and Hemoproteins," ed. by B. Chance, R. W. Estabrook and T. Yonetani, Academic Press, New York (1966), p. 9.

[14] M. F. Perutz, *Scientific American*, **211**, 5 (1964). J. C. Kendrew, *Science*, **139**, 1259 (1963).

[15] J. L. Hoard, M. J. Hamor and T. A. Hamor, *J. Amer. Chem. Soc.*, **85**, 2334 (1963).

[16] S. Silvers and A. Tulinsky, *ibid.*, **86**, 927 (1964).

[17] C. Weiss, H. Kobayashi and M. Gouterman, *J. Mol. Spectrosc.*, **16**, 415 (1965).

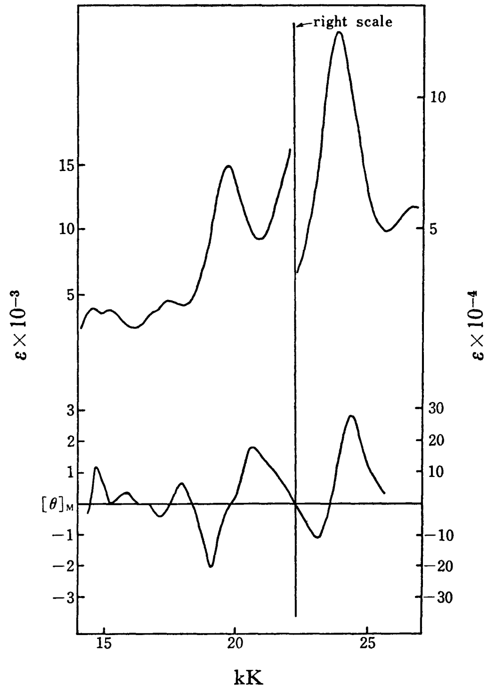

Fig. 2. MCD and absorption spectra of TPPFe(III)-Cl in benzene.

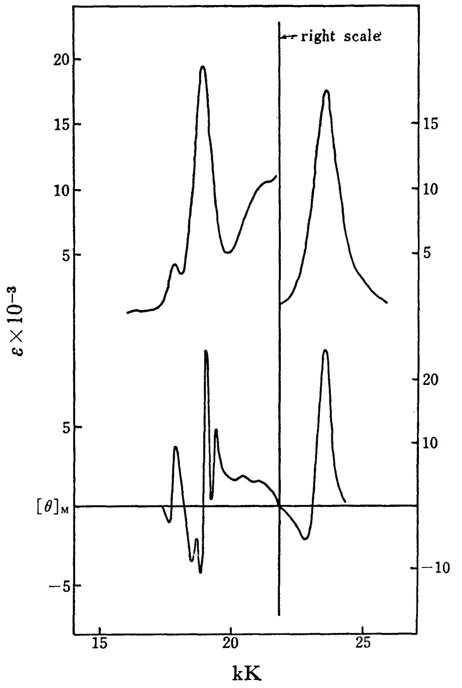

Fig. 4. MCD and absorption spectra of TPPFe(II)·2Py in pyridine.

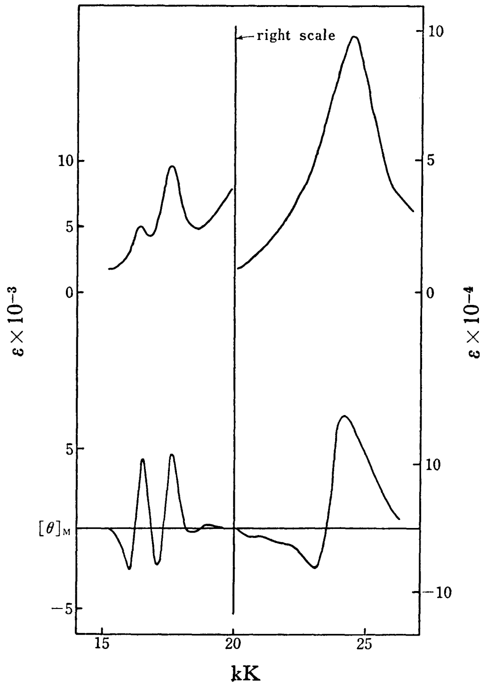

Fig. 3. MCD and absorption spectra of TPPFe(III)-OCH_3 in benzene.

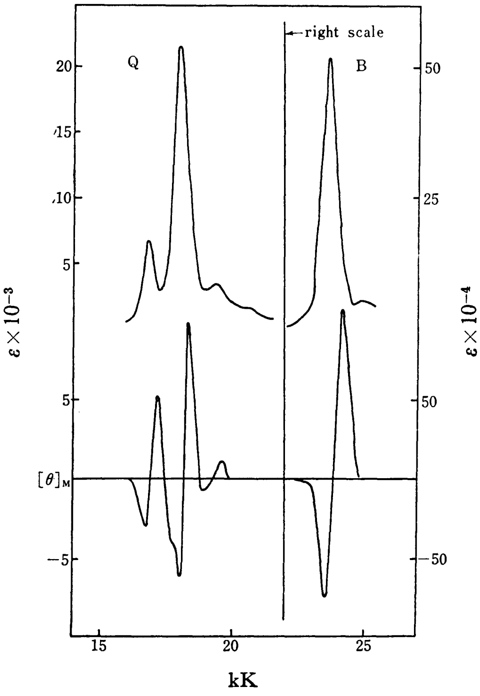

Fig. 5. MCD and absorption spectra of TPPZn in THF.

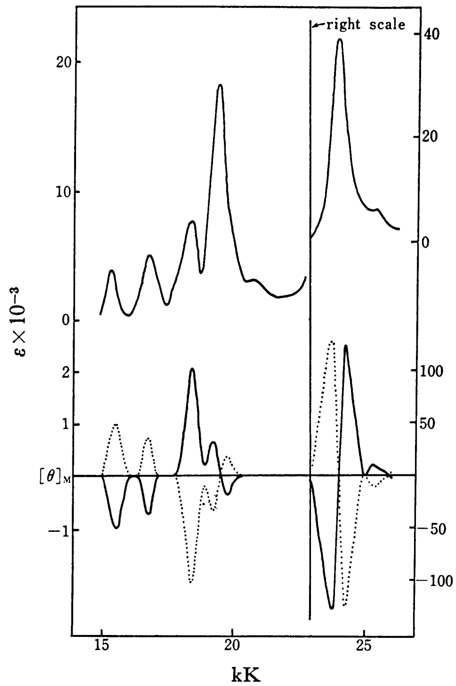

Fig. 6. MCD and absorption spectra of the metal free base of tetraphenylporphin in THF. MCD spectra are given for a normal magnetic field (———) and a reversed magnetic field (·········). The sense of the normal magnetic field was set to that of the light beam propagation.

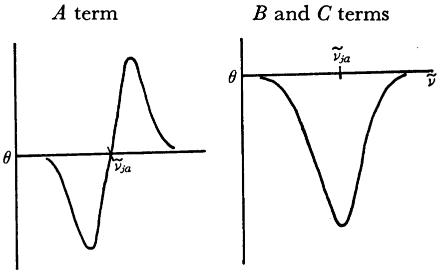

Fig. 7. The band shapes of MCD and absorption spectra.

$$B=\frac{3}{d_a}\sum I_m\Big\{\sum_{k\neq a}\frac{\langle k|\hat{\mu}_z|a\rangle}{W_k-W_a}[\langle a|\hat{m}_x|j\rangle\langle j|\hat{m}_y|k\rangle -\langle a|\hat{m}_y|j\rangle\langle j|\hat{m}_x|k\rangle] +\sum_{k\neq j}\frac{\langle j|\hat{\mu}_z|k\rangle}{W_k-W_j}[\langle a|\hat{m}_x|j\rangle\langle k|\hat{m}_y|a\rangle -\langle a|\hat{m}_y|j\rangle\langle k|\hat{m}_x|a\rangle]\Big\} \tag{3}$$

$$C=\frac{3}{d_a}\sum\langle a|\hat{\mu}_z|a\rangle I_m[\langle a|\hat{m}_x|j\rangle\langle j|\hat{m}_y|a\rangle] \tag{4}$$

W_a, W_k and W_j are the energy of the respective states, d_a is the degeneracy of the state a, and $\hat{m}$ and $\hat{\mu}$ are the electric and magnetic dipole operators, respectively.

f_1 is a function which shows a dispersion at the absorption maximum, while f_2 is a function which shows a maximum or a minimum at the absorption maximum. If term A is predominant, $[\theta]_M$ shows an anomalous dispersion at the absorption maximum, while if term B or C is predominant, $[\theta]_M$ shows an extreme value as shown in Fig. 7. Term A depends upon the absorption intensity and also the difference of angular momenta between the ground state and the excited state involved in the absorption. If the ground state is not degenerate, term A depends on the angular momentum only of the excited state. Term C depends upon the angular momentum of the ground state. Term B is ascribed to the angular momenta induced by the external magnetic field. As seen in Fig. 5 the MCD spectrum of TPPZn(II) is predominantly described by term A. As has been shown by the molecular orbital calculations,[17] the lowest (π,π^*) excited state (^1Q state) of porphin is of E_u symmetry and has an angular momentum of ±9 in the sense of a circular box model, while the second one (^1B state) which gives rise to the Soret band, is also of E_u symmetry and has an angular momentum of ±1. While in the case of the metal free base, the degenerate excited states of D_{4h} porphin assumed for the metalloporphins such as TPPZn(II) are split by the localization of the central two protons to the nitrogen atoms at opposite positions.[16,18] The splitting of the lowest degenerate transition in the visible region is fairly large, while that of the second one in the near-ultraviolet is usually too small to be observed. Therefore, as seen in Fig. 6 the MCD spectrum in the visible region of the metal free base should show a contribution of term B, mainly arises from the magnetically induced mixing between the split components of the degenerate excited states. The MCD observed in the near-ultraviolet region can be ascribed to term A. The ground state of TPPZn(II) and also of the metal free base can be described by a closed shell configuration, term C being vanished.

In the case of ferric porphin, the absorption spectra in the visible- and near-ultraviolet region can not be described in terms of (π,π^*) transitions, because of appreciable configuration mixings due to the charge-transfer excited states of exactly the same symmetry, E_u. The charge-transfer excited state arises from the charge-transfer from one of the top filled π molecular orbitals of porphin to one of the vacant degenerate $d\pi$ orbitals. There seem to be several excited states in the visible- and near-ultraviolet region other than the ^1Q and ^1B states which are ascribed to the (π,π^*) excitations. Thus the contribution of term B due to the magnetically induced mixings between the closely existing states is expected for the MCD of these complexes. Furthermore $\mathrm{TPPFe(III)OCH_3}$ is expected to have an

---

[18] J. Weigl, *J. Mol. Spectrosc.*, **1**, 133 (1957); B. G. Anex and R. S. Umans, *J. Amer. Chem. Soc.*, **86**, 5026 (1964).

angular momentum due to the orbital motion of a hole in the $d\pi$ orbitals in the ground state, while TPPFe(III)Cl lacks such an angular momentum. Although further experimental work under various temperatures is required to see how it depends on each of terms *B* and *C*, the MCD observed for the high-spin TPPFe(III)Cl and the low-spin TPP-Fe(III)OCH_3, as shown in Figs. 2 and 3, can be ascribed to a contribution of the terms *B*, *C* as well as *A*.

In the case of diamagnetic ferrous porphin, TPP-Fe(II)·2Py, the absorption spectrum in the visible- and near-ultraviolet region was very close to that of TPPZn(II). However, the MCD were quite different. A predominant contribution of term *B* to the MCD was observed at the Soret band and also at the 21000 cm^{-1} band. The latter can be assigned to a charge-transfer transition of a $d\pi$ electron of the iron to a vacant pyridine molecular orbital, since the piperidine adduct of TPPFe(II) does not show the 21000 cm^{-1} band.[8] Similarly near the Soret band there exist charge-transfer excited states which produce a hole in the closed $d\pi$ orbitals. These charge-transfer excited states are mixed to the Soret band by the external magnetic field. In oxyhemoglobin and carboxyhemoglobin, the iron porphyrin exists in a diamagnetic state. Their MCD and absorption spectra are given in Figs. 8 and 9. The absorption intensity of the iron porphyrin in these proteins was slightly weaker than that of TPPFe(II)·2Py, while the MCD of the iron

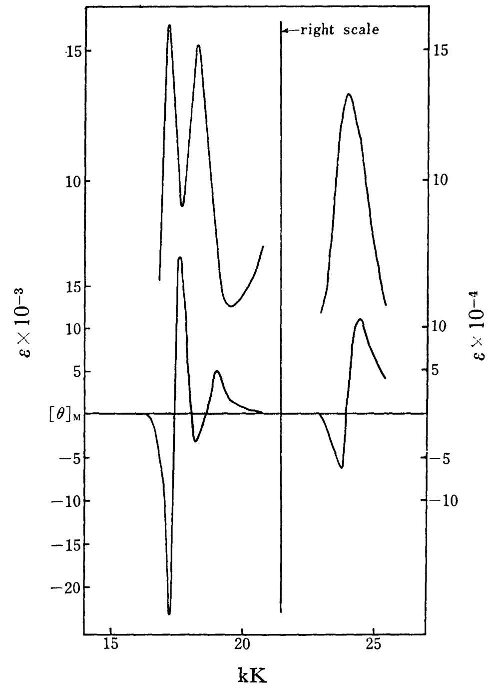

Fig. 9. MCD and absorption spectra of oxy-hemoglobin.

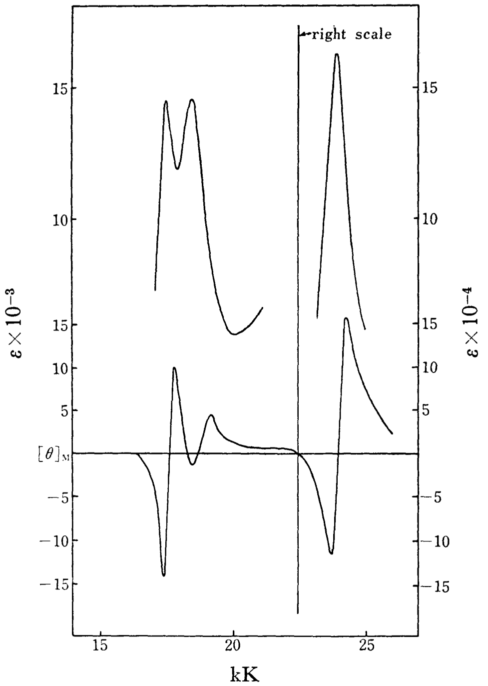

Fig. 8. MCD and absorption spectra of carboxy-hemoglobin.

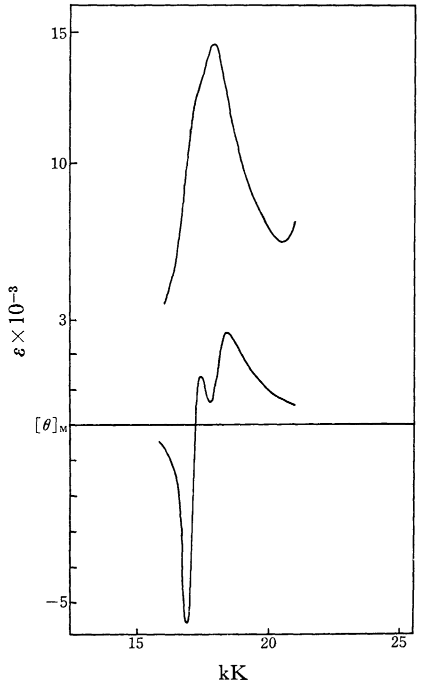

Fig. 10. MCD and absorption spectra of deoxy-hemoglobin.

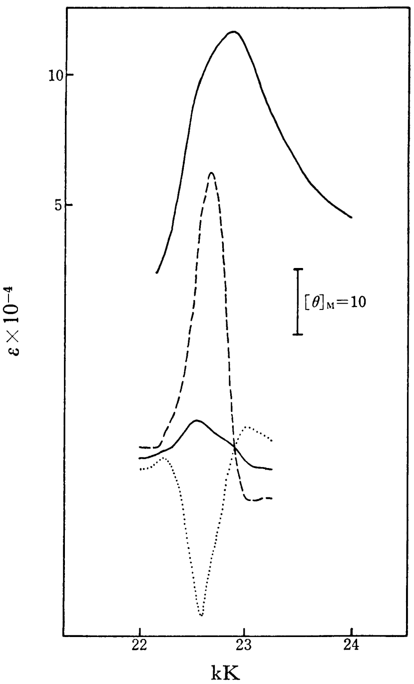

Fig. 11. A non-linear relationship between the MCD and the CD in deoxyhemoglobin.
———: Natural CD.
- - - -: MCD observed in a normal magnetic field (The sense of the magnetic field is that of the light beam propagation).
·········: MCD observed in a reversed magnetic field.

porphyrin in these proteins was much stronger. In these naturally occurring proteins, there exists a circular dichroism due to the helical structure of the counterpart of the iron porphyrin. Assuming an additivity between MCD and CD, the difference between observed CD values for the magnetic and non-magnetic conditions was assigned to the MCD of these proteins. The iron(II) porphyrin in these proteins seems to be quite free from the contribution of term *B* to the MCD at the Soret band. This indicates the charge-transfer excited states, which show an angular momentum due to a hole produced in the closed $d\pi$ shell, are fairly higher than that of TPPFe(II)·2Py.

The MCD and absorption spectra of deoxyhemoglobin are shown in Figs. 10 and 11. When the current in the electromagnet was reversed, the deviation between the circular dichroisms in the near-ultraviolet region for the magnetic and non-magnetic conditions was not symmetrically reversed in this particular case (Fig. 11). There might be a non-linear interaction between MCD and CD. The MCD of deoxyhemoglobin remarkably shows a contribution of terms *B* and *C*.

The authors wish to thank Dr. T. Samejima, Aoyama Gakuin University for kindly permitting them to use his instrument at a preliminary stage of this work. One of the authors (H.K.) is grateful to Professor S. Nagakura, The University of Tokyo for his interest in the work. Grateful acknowledgment is also made to Dr. M. Shigehisa of Japan Spectroscopic Co. Ltd. for helpful technical advice. This research was supported by a grant from the Ministry of Education.