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CD SPECTRA OF DEUTEROHEMIN DERIVATIVES WITH ONE OR TWO IMIDAZOLE(S) COVALENTLY BOUND TO THE PORPHYRIN RING

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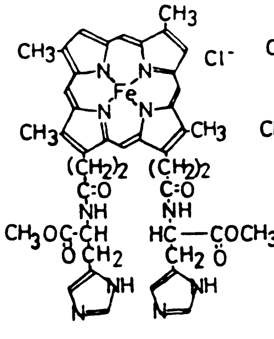
Origins of the Soret optical activity were investigated by CD spectra of the compound described in the title. The chelation of the axial ligand group(s) was demonstrated to be an important factor for the large optical activity observed on the Soret region which was critically dependent on the spin state of the heme iron.

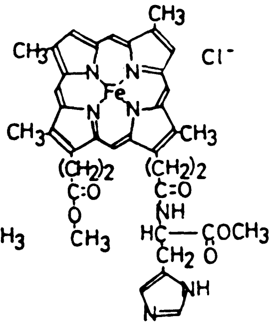
Circular dichroism(CD) has high potential for researches on conformation of proteins. Many investigations for hemoproteins which contain heme derivatives as prosthetic groups have been reported.[1-6)] However, there is no CD report about a low molecular weight model compound, except for the one on the heme c systems which still contain some peptide residues.[7-9)]

The deuterohemin derivatives which we used in this study contain one or two L-histidine methyl ester(s) covalently bound to the porphyrin ring. Since these deuterohemins have no extraneous protein residue, they are expected to be very simple models to explore the origins of the heme optical activity. We wish to report preliminary results of the CD spectral studies on these deuterohemin derivatives.

Hemins I and II which have one and two imidazole side chain(s), respectively, were synthesized through the reaction of deuterohemin with L-histidine methyl ester and purified by column chromatographies.[10)] CD spectra were recorded on a JASCO Model J-500C spectrodichrometer. Absorption spectra were obtained with a JASCO UVIDEC-510 spectrophotometer. All measurements were carried out in methanol at the hemin concentration of about 10^{-4} mole dm^{-3}. At this concentration any aggregation of the hemin derivatives does not occur because there was linear relationship between the molar absorption coefficient and the concentration of the hemin.

Figure 1 shows CD and absorption spectra of Hemin I without and with sodium hydroxide in the molar ratio of 4:1 to the hemin. Since Hemin I has two imidazoles as axial ligands, ferric heme iron is of low spin. When sodium hydroxide was added to the methanol solution of Hemin I, the absorption spectrum changed from a low spin type to a high spin type with two isosbestic points. This suggests that


Hemin I


Hemin II

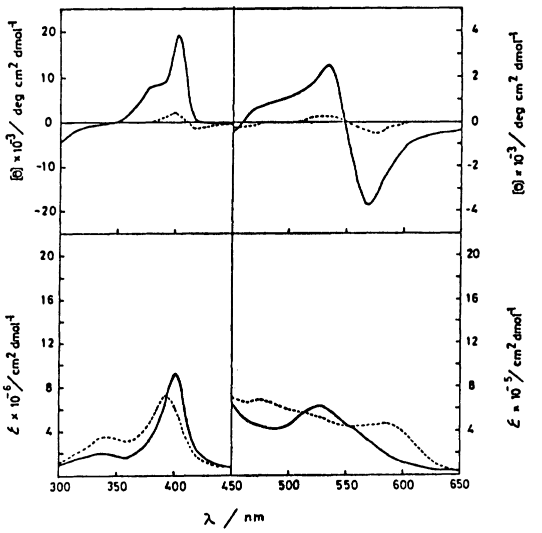


Fig. 1. CD(upper) and absorption(lower) spectra of Hemin I (———) and Hemin I with NaOH (1:4) (-----) in methanol at the hemin concentration of 1.21 x 10^{-4} mole dm^{-3}.

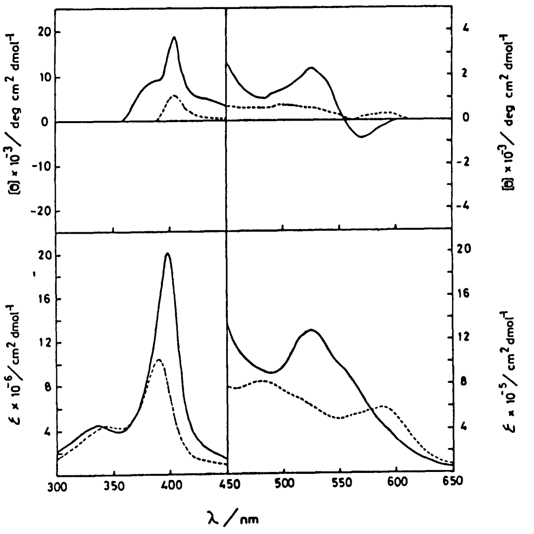


Fig. 2. CD(upper) and absorption(lower) spectra of Hemin II (-----) and Hemin II with imidazole (1:250) (———) in methanol at the hemin concentration of 7.74 x 10^{-5} mole dm^{-3}.

one of the two axial imidazoles was substituted by a hydroxide ion and then the heme iron changed to a high spin state.

Figure 2 shows CD and absorption spectra of Hemin II without and with excess imidazole. Since the iron of Hemin II is of high spin, Hemin II would have one imidazole molecule and one methanol molecule (or the counter ion, Cl^-) as axial ligands. By the addition of large excess of imidazole, the coordinated methanol molecule (or Cl^-) will be replaced by one imidazole molecule, and then, the spin state of the heme iron changed from high to low.

Figure 3 shows CD and absorption spectra of deuterohemin dimethyl ester with large excess amounts of BOC-L-histidine methyl ester, and Hemin I with large excess amounts of hydrochloric acid. Deuterohemin dimethyl ester must have two imidazoles as axial ligands from BOC-L-histidine methyl ester, since the heme iron is of low spin. While, since the iron of Hemin I is of high spin, the imidazoles of the side chains did not coordinate to the heme iron due to a protonation.

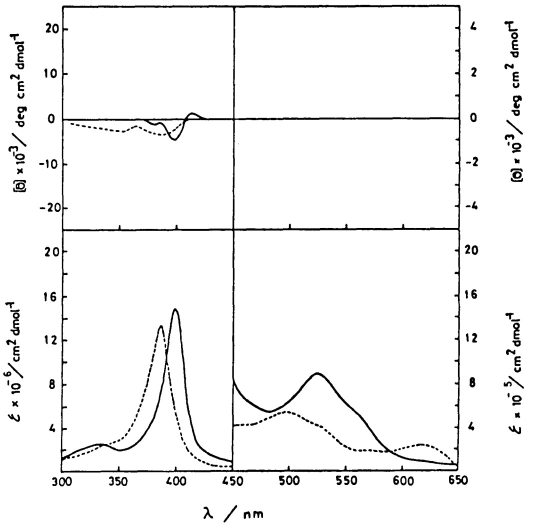
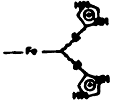
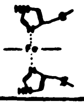


Fig. 3. CD(upper) and absorption(lower) spectra of deuterohemin dimethyl ester with BOC-L-histidine methyl ester (1:2300) at the hemin concentration of 1.21 x 10^{-4} mole dm^{-3} (———) and Hemin I with HCl (1:100) at the hemin concentration of 9.92 x 10^{-5} mole dm^{-3} (-------).

The numerical data of the Soret absorption and CD bands are summarized in Table 1 which also includes the spin states and the complex structures expected by absorption spectra. Main CD bands for samples of Nos. 3 and 6 are small negative (see also Fig. 3), while for the others positive. Since the latters have at least one imidazole chelation, the chromophores in the chelate chain (imidazole and carbonyl group of the amide bond) may be fairly fixed against the heme plane. The formers, on the other hand, do not have the chelate ring, and then the axial ligands rotate freely. The optical activity of the hemin derivatives is thought to originate from the relative spatial relation between the porphyrin ring and the chromophores of the side chain which contains one asymmetric carbon. Therefore, the chelate effect is expected to be a significant factor on the Soret optical activity in these model systems. The comparison of Nos. 4 and 5 demonstrates that for the hemin derivatives with one imidazole chelate ring the low spin species exhibited larger optical activity than the high spin one did. This suggests that the spin state of the heme iron is important for the Soret optical activity. Such an observed clear dependence of the optical activity on the spin state may originate from the structural factors rather than mere electronic ones. Therefore, the relative orientation of the chromophores of the side chain to the heme plane could be more flexible in the high spin state than in the low spin one. This may be closely related to the position of the heme iron. Thus, as well-known, the heme iron is displaced from the heme plane by about 0.5 Å in the high spin state.[11)]

In the last column of Table 1, the anisotropy factors, $[\theta]_{max}/\varepsilon_{max}$, are given in the unit of degree. Using these values, the contribution of the several factors for the Soret optical activity is able to be estimated. The values of the anisotropy factors of Nos. 1 and 5, which are in a low spin state and have two and one imidazole chelate ring(s), are about $2\text{x}10^{-3}$ and $1\text{x}10^{-3}$, respectively. Therefore, the contribution of one chelate ring for the anisotropy factor of the low spin

Table 1. The numerical data of the Soret absorption and CD bands of the deuterohemin derivatives in methanol.

NO.	Complexes	Spin states	εmax. (nm)* $\times 10^{-6}$cm^{2}dmol^{-1}	[θ]max. (nm)* $\times 10^{-3}$deg cm^2 dmol^{-1}	[θ]max./εmax. $\times 10^3$deg
1		low spin	9.32 (401)	19.06 (402)	2.05
2	 a)	high spin	7.17 (393)	2.02 (400)	0.28
3	 b)	high spin	13.32 (387)	-3.21 (386)	-0.24
4		high spin	10.43 (390)	5.64 (405)	0.54
5	 c)	low spin	20.06 (398)	18.76 (405)	0.94
6	 d)	low spin	14.94 (400)	-4.88 (399)	-0.33

a) [hemin] : [NaOH] = 1 : 4, b) [hemin] : [HCl] = 1 : 100, c) [hemin] : [imidazole] = 1 : 250, d) [deuterohemin dimethyl ester] : [BOC-L-histidine methyl ester] = 1 : 2300.
*Wavelengths in nm are described in the parentheses.

hemins is estimated to be about 1×10^{-3}. In the high spin state, on the other hand, the effect of one imidazole chelate ring is evaluated to be about 0.5×10^{-3} from No. 4. Thus, the effect of the imidazole chelate ring in the low spin hemins is thought to be about twice that in the high spin ones. From No. 3, one free side chain seems to contribute to the anisotropy factor by about -0.1×10^{-3}. Then, the value of No. 2 which contains one chelate ring and one free side chain is reasonably interpretable from the values estimated above. Consequently, the contributions of the imidazole chelate ring and the spin state of the heme iron on the optical activity can be grasped somewhat quantitatively.

The results described above differ in some points from those found in the heme *c*-peptide systems by Urry7,8) and Pande.et al.9) They showed that (1) when identical ligands coordinated at the axial positions, irrespective of the oxidation state and the spin state of the heme iron, a small negative CD band was observed in the Soret region, and (2) when one axial ligand was the imidazole of histidine-18, CD depended on the nature of the sixth ligand group; the large CD band for the weak ligand field group such as H_2O and the small CD for the strong group such as imidazole. Myer and Pande12) attributed these phenomena to the lowering of the symmetry of the heme group. In our systems, on the other hand, a strong field ligand as the sixth ligand favors large optical activity for the Soret band more than a weak field ligand does, and the lowering of the symmetry due to the difference of the two axial ligands rather shifted the Soret CD peak to longer wavelength as seen from Nos. 2, 4, and 5.

These differences between our models and Urry's may be partly due to the existence of the ring with optically active centers in the case of heme *c*-peptide. Since our deuterohemin systems are much simple with no extra optically active peptide, we were able to estimate the factors on the heme optical activity.

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