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MAGNETIC CIRCULAR DICHROISM AND SPIN EQUILIBRIUM OF METHEMOGLOBIN AND ITS SUBUNITS

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SUMMARY: The intensity of the Soret magnetic circular dichroism $(MCD)_+$ spectra of various complexes of methemoglobin subunits (α^+ and β^+) as well as methemoglobin (metHb A) was correlated well with the spin states of ferric heme. Upon the subunit association, spin state transition toward higher spin was observed only in high spin derivatives and the changes in spin state were due to mainly those of β^+ chains. The effect of an allostric effector, inositol hexaphosphate (IHP), on the MCD spectra of metHb A derivatives was observed much significantly for high spin forms than low spin ones.

Methemoglobin A (MetHb A) is stable but its subunits, α^+ and β^+ chains, are very unstable compared with ferrous subunits even in the presence of 1M glycine at $0^{\circ}C$, which has been often used for stabilization of ferric chains (1). This fact indeed delayed the detailed study on the molecular properties of ferric chains. Recently, we measured the optical properties (absorption and circular dichroism spectra (CD)) of the ferric heme derivatives of α^+ and β^+ chains. Major results obtained were that the dramatic changes in the Soret CD spectra were observed when $\alpha_1\beta_1$ dimer was formed by the association of α^+ and β^+ chains, on the other hand, such changes in the Soret absorption spectra were not observed (2). Since there are little reports dealing with the magneto-optical properties of a series of high and low spin derivatives of α^+ and β^+ chains and changes in the magnetic properties of α^+

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Abbreviations: α^+ , ferric α chain; β^+, ferric β chain; bis-Tris, 2-(bis(2-hydroxyethyl)amino)-2-(hydroxymethyl)-propane-1.3-diol.

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and β^+ chains upon the subunit association, we measured the Soret MCD spectra of the ferric chains and metHb A. We also examined the effect of binding of an allosteric effector, inositol hexaphosphate (IHP), to various derivatives of metHb A on the MCD spectra.

MATERIALS AND METHODS: Stripped human adult hemoglobin (Hb A) and its subunits were prepared from fresh adult blood (4,5). MetHb A was obtained by oxidation of oxyHb A with 3 molar amounts of ferricyanide, and an excess of ferricyanide and ferrocyanide produced were removed by passage through a Dowex 1x8 column. Ferric chains were obtained as in (1). Absorption spectra were measured with a Union SM 401 spectrophotometer and expressed in terms of millimolar extinction coefficient, ε_{mM} ($mM^{-1}cm^{-1}$). MCD spectra were recorded on a JASCO J-20 spectropolarimeter equipped with a permanent magnet of 4500 gauss, and expressed as molar ellipticities on a heme basis per gauss ($deg.cm^2/dmole/gauss$). Heme concentration was determined by pyridine hemochromogen method using a millimolar extinction coefficient of 34 at 557nm. All chemicals used were of analytical grade, and were used without further purification.

RESULTS AND DISCUSSION

Fig.1 shows the MCD and absorption spectra in the Soret region of various complexes of α^+ and β^+ chains in 1M glycine, pH 7.0, at 0^oC. In each complex, the Soret MCD spectral feature of α^+ chains was very similar to that of β^+ chains. Although the MCD intensity of α^+ chains was different from that of β^+ chains in all derivatives, the MCD intensity of both chains was increased in the order, $F^- < H_2O < N_3^- < CN^-$. This result suggests that the Soret MCD intensity of the isolated ferric chains is proportional to the spin state of ferric iron, as observed previously in metMb (6). The Soret absorption intensity of α^+ chains was always greater than that of β^+ chains in all derivatives (Fig.1). The MCD spectrum measures the difference in absorption extinction coefficient between right and left circularly polarized light under magnetic field. Therefore, the MCD intensity may also depend on the intensity of the absorption spectrum, so that the ratio of the MCD intensity (defined as the distance between the

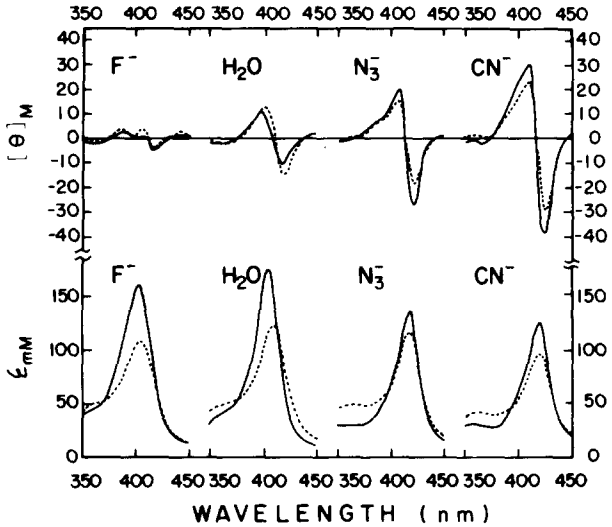

Fig.1 The MCD (upper) and absorption (lower) spectra in the Soret region of various complexes of α^+ (——) and β^+ (---) chains measured in 1M glycine, pH 7.0, at 0°C. Heme concentration was 60 μM as the heme basis. Ferric ligand concentration: 0.1M NaF for F^-; 1mM NaN_3 for N_3^-; 1mM NaCN for CN^-.

MCD peak and trough) to the intensity of the absorption maximum, ($[\theta]_M/\varepsilon_{mM}$), would correlate with the spin state. However, the magnetic susceptibility of α^+ and β^+ chains has not been reported because of the instability of the chains at high concentration required for the measurements even in the presence of 1M glycine (7). In metMb and metHb derivatives, it has been shown that the peak position of the Soret absorption band correlates with the magnetic susceptibility (8,9). This suggests that a certain relation may also exist between the Soret MCD intensity and absorption peak position of metHb A. Fig.2 shows the relation between the ratio, ($[\theta]_M/\varepsilon_{mM}$) and the peak position for metHb A. Fig. 2 also shows that the relation holds for α^+ and β^+ chain derivatives as well, strongly suggesting that the MCD intensity of the isolated ferric chain derivatives correlates well with the magnetic susceptibility.

Fig.3 shows the MCD spectra in the Soret region of metHb A and the simple addition of those of α^+ and β^+ chains ($(\alpha^+ + \beta^+)/2$).

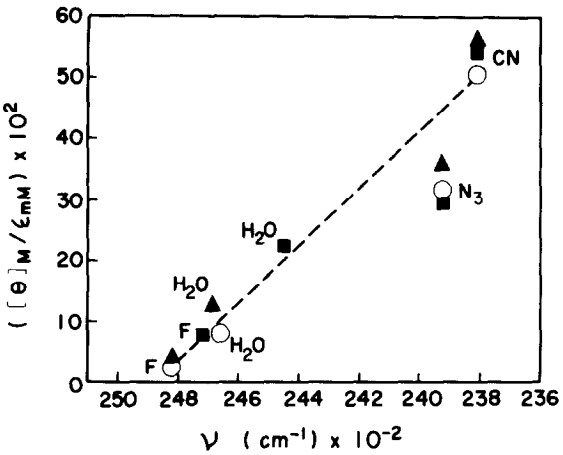

Fig.2 Correlation between the Soret MCD intensity and peak position of absorption maximum of metHb A and its subunits.
○ : metHb A; ▲ : α^+ ; ■ : β^+

The MCD spectra of metHb A were almost identical with the sum of those of the isolated chains in low spin forms, whereas in high spin forms the mean spectra of α^+ and β^+ chains were significantly different from those of metHb A. These results indicate that

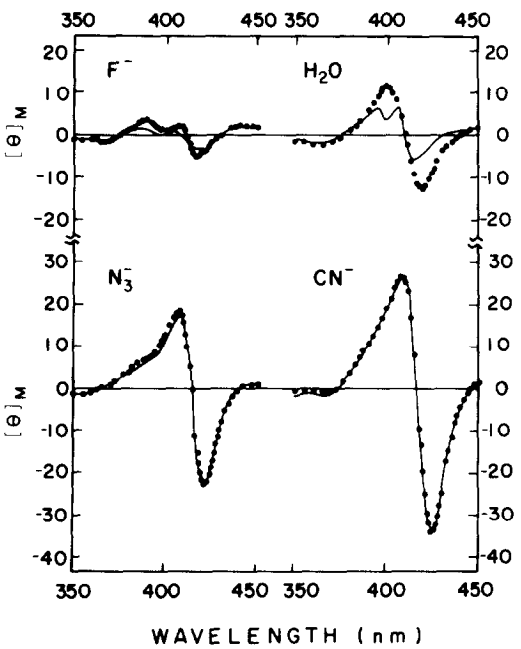

Fig.3 Effect of subunit association on the Soret MCD spectrum.
——— : metHb A ; ••••• : ($\alpha^+ + \beta^+$)/2
Experimental conditions are as in Fig.1.

subunit association had little effect on the spin state in low spin forms as suggested by NMR study (10); however, in high spin forms spin equilibrium was shifted toward higher spin by subunit association. Banerjee et al. (11,12) reported for aquomet form that low spin content in β^+ chains is larger than that in metHb A and α^+ chains, and that the increased low spin character of β^+ chains disappear upon association with β^+ chains. Neya and Morishima (10) observed similar results for high spin derivatives by NMR, except for the fluoride form. As shown in Fig.2, in fluoride and aquomet forms α^+ chains are near metHb A, but β^+ chains are distinctly separated from metHb A and α^+ chains. These results suggest that not only in aquomet form but also in fluoride form the low spin content in β^+ chains is substantially larger than that in α^+ chains and metHb A, and that the changes in MCD intensity induced by subunit association result mainly from those of β^+ chains. The increased low spin character of β^+ chains in high spin forms may arise from stronger interaction between heme iron and proximal histidine in β^+ chains than in α^+ chains, and the interaction of β^+ chains may be weakened upon association with α^+ chains (2,10,12).

It has been known that high spin forms of metHb A are converted to the T state by IHP, but low spin forms remain in the R state even in the presence of IHP (13,14). IHP caused a red shift of the Soret absorption band in metHbA(F^-), no shift of this band in metHb A(H_2O) at pH 7.0, a blue shift in metHb A(N_3^-), and a slight red shift in metHb A(CN^-) (13). Upon the addition of IHP, a rise in magnectic susceptibility was observed for metHb A(H_2O) and metHb A(N_3^-) (13,14). These results suggest that although the peak position of the Soret band correlates with the spin state of ferric heme as shown in Fig.2, changes in the Soret peak position are not always reflected to changes in magnetic susceptibility,

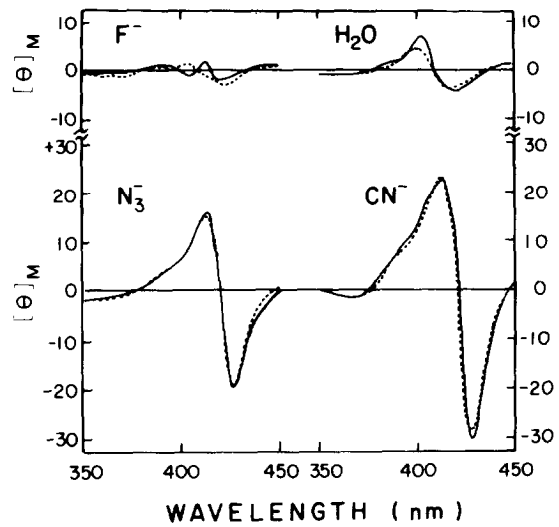

Fig.4 Effect of IHP on the Soret MCD spectrum of metHb A derivatives.
——— : - IHP; ------ : + 1mM IHP.
Samples were in 0.05M Bis-tris in 0.1M NaCl, pH 7.0, at 25°C. Other conditions are as in Fig.1.

as suggested by Perutz et al. (14) for the visible absorption spectrum that has been used for monitoring spin equilibrium. Therefore, it is of interest to examine the effect of IHP on the Soret MCD spectra of metHb A derivatives.

Fig.4 shows the Soret MCD spectra of metHb A derivatives in the presence and absence of IHP. Upon the addition of IHP, changes in MCD spectral shape were observed for the fluoride form, but the intensity of MCD was distinctly decreased for the aquomet form. Such small decreases in the intensity were also observed for low spin forms. The decrease in MCD intensity for aquomet- and azidemet-Hb A is quite consistent with the rise in magnetic susceptibility (13,14) and reflects the shift in thermal spin equilibrium toward high spin by IHP. X-ray analysis for metHb A(F^-) bound with IHP showed that the iron atom of α heme is either located in the heme plane or dislocated up to 0.8 Å on the distal side, and suggested the possibility of rupture of the bond between iron and the proximal histidine (15). Therefore, changes

in MCD spectral shape of metHb A(F^-) by addition of IHP may arise from such alteration in the heme environment. Although the rise in magnetic susceptibility by IHP binding had not been detected in metHb A(CN^-) (13), the small decrease in MCD intensity seems to reflect the alteration in the spin state by IHP due to changes in free energy difference between the high and low spin states.

The above results demonstrate that the Soret MCD intensity depends on low spin content of ferric heme, and that changes in MCD intensity in turn reflect an alteration in the spin state. Accordingly, the Soret MCD spectra are considered to be a useful means not only for studying the spin state, but also for monitoring changes in spin equilibrium of the ferric heme in metHb A caused by some allosteric effectors.

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