CONFORMATIONAL STATE AND R⇄T TRANSFORMATION IN MN(II) AND MN(III) HEMOGLOBINS AND AZIDE MN(III) HEMOGLOBIN

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SUMMARY

The circular dichroism of Mn(II) and Mn(III) hemoglobins has been measured in the uv and Soret spectral regions, and the differences are indicative of an R⇄T allosteric transformation, particularly in the 280 nm region. However, Mn(III) hemoglobin in the uv region is distinctly different from aquomet hemoglobin. The addition of inositol hexaphosphate to Mn(III) hemoglobin gives a uv circular dichroic spectrum characteristic of the T form. In contrast to the azide derivative of Fe(III) hemoglobin, the azide derivative of Mn(III) hemoglobin is converted to the T form by the addition of inositol hexaphosphate.

INTRODUCTION

The molecular conformational changes associated with the cooperative binding of oxygen to hemoglobin (Hb) are of considerable importance to the determination of the mechanism of oxygenation. The "trigger mechanism" correlates the position of the iron atom relative to the heme plane with oxygenation and conformational state of the molecule, i.e., Fe(II) in heme plane (six-coordinate) when oxygenated and R molecular conformation; Fe(II) (five-coordinate) out of the heme plane toward histidine F(8) and T conformation when deoxygenated (1,2). The conformational state of the tetramer (R,T) can be probed spectrally and, in particular, by circular dichroism (CD) in the uv and in the Soret regions (3). In the 280 nm region a weak positive ellipticity is associated with the R form whereas a large negative ellipticity is associated with the T form. The Soret region also undergoes a change in rotational strength upon the R⇄T conformational change (4). One method of studying the effect of the metal environment on the protein conformation is by metal substitution such as cobalt, manganese and other transition metals since they generally have different stereochemical requirements than iron.

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Cobalt hemoglobins (coboglobins) bind oxygen reversibly and undergo the R⇄T allosteric transformation (5,6). The CD spectra of oxy, deoxy Co(II) have been examined by Chien and Snyder (7). In the 280 nm region they found that oxy Co(II) has a spectrum indicative of the R conformation and deoxy Co(II)Hb has a spectrum characteristic of the T conformation. Co(II)Hb is always low spin regardless of whether the molecule is in the oxy or deoxy form. Co(III)Hb, on the other hand, appears to be a low spin internal hemochrome, that is, the metal (Co(III)) is constrained (rigid) in a six-coordinate environment which in contrast to aquomet Hb (8), is invariant to the addition of IHP.

In contrast to the coboglobins, Mn(II)Hb and Mn(III)Hb are always high spin(with the possible exception of nitrosyl Mn(II)Hb) (9). Model compound studies have not only shown that the Mn(III) environment can change from five to six coordinate with accompanying changes in metal-ligand axial distances and shrinking metal to mean plane distances (10), but also the metal-axial ligand distance can change with appropriate ligands even comparing six coordinate Mn(III) compounds (11); i.e., the metal ion environment is flexible. Therefore, if the metal ion geometry is intimately linked (Perutz's trigger (1,2)) to the conformational state of the tetramer it should be possible to change the quaternary conformation of Mn(III)Hb by appropriate additions. Although manganese hemoglobin does not bind oxygen, allosteric effects have been observed in oxidation equilibria and kinetics of ligand binding (12,13). The X-ray structure results of Moffatt, et al., on Mn(III)Hb seem to be at variance with the uv difference spectra of Hoffman, et al., who showed that the difference spectra of aquomet Hb with and without added IHP was the same as that of Mn(III)Hb with and in the absence of IHP (14,15). This latter result implies that stripped aquomet Hb and Mn(III)Hb have the same conformation (R). This apparent conflict of data and our development of new synthetic techniques to make metalloporphyrins in high yield and use of earlier CD results led us to examine the CD of the Mn(II)Hb and Mn(III)Hb's in the 280 nm region.

MATERIALS AND METHODS

Protoporphyrin IX dimethyl ester was converted to the acid form by stirring overnight in 6M HCl at 4°. The solution was then brought to pH 4.0 with 10M NaOH, filtered and washed free of chloride ions with distilled water and vacuum dried. The manganese protoporphyrin complex was prepared by reacting protoporphyrin IX (30 mg) with manganese acetate (30 mg) in N,N-dimethylformamide (40 ml). The reaction mixture was heated at refluxing temperature (150°C) for 10-15 min., allowed to cool and filtered. Pure protoporphinatomanganese(III)·DMF (50% yield based of porphyrin) was obtained upon removal of the DMF solvent. Titration of apohemoglobin with Mn(III) protoporphyrin IX was done in 0.1M potassium phosphate, pH 7.0, monitoring

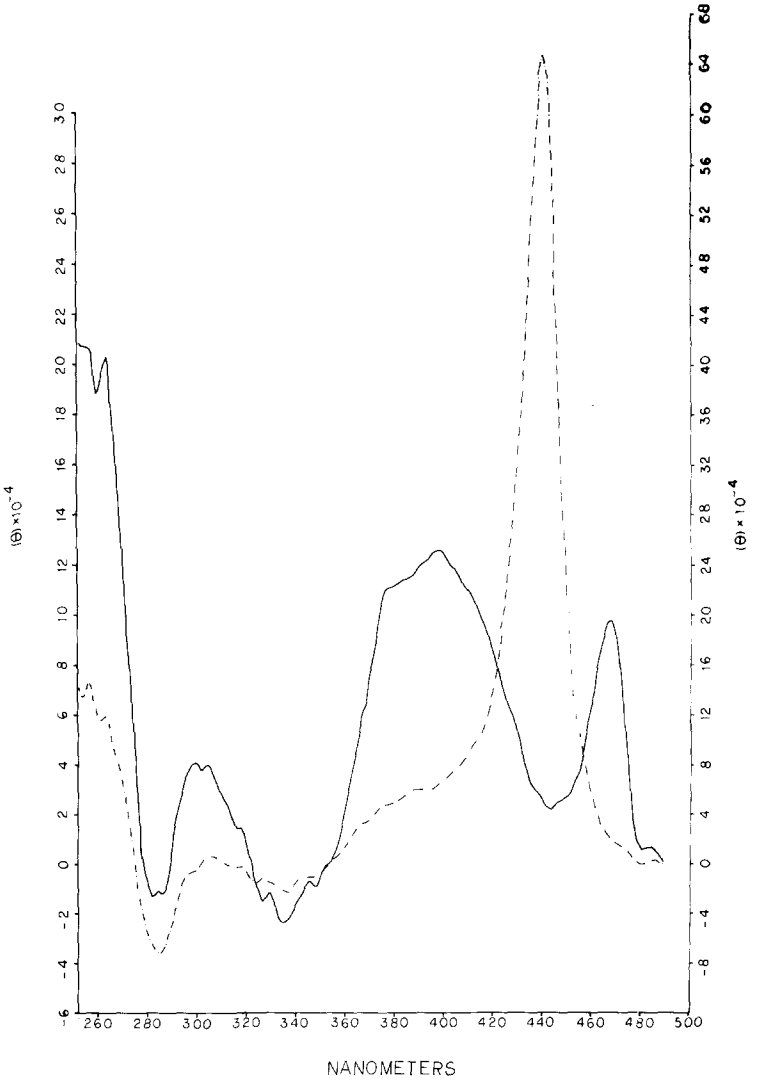

Figure 1: CD spectra of a 24 μM (tetramer) solution of Mn(II) and Mn(III) hemoglobin in 50 mM bis-TRIS of pH 6.5. (---) Mn(II) hemoglobin, (——) Mn(III) hemoglobin. Ellipticities are reported in terms of tetramers. The right ordinate refers to Mn(II) hemoglobin.

the increase in absorption at 467 nm as described by Waterman and Yonetani (16).

Apohemoglobin was prepared by the acid butanone method (17). Mn(III) hemoglobin for CD studies was prepared by addition of a 1.3-fold excess of Mn(III) porphyrin in 0.1M NaOH to the apohemoglobin in 50 mM bis-Tris pH 7.0. The solution was allowed to stand overnight at 4° and the excess Mn(III) porphyrin was removed by chromatography on Sephadex G-10 equilibrated with 50 mM bis-Tris of pH 6.5. [All phases of the preparation of Mn(III) hemoglobin for CD studies were done at 4°.]

Mn(II) hemoglobin was prepared from Mn(III) hemoglobin by addition of a 1.2-fold excess (mole/mole of porphyrin) of sodium dithionite to the Mn(III) hemoglobin in 50 mM bis-Tris pH 6.5. Sodium dithionite solution was prepared in a nitrogen filled glove box by diluting the dithionite to a known concentration with 50 mM bis-Tris pH 6.5. Approximately one hour was allowed for the conversion of Mn(III) hemoglobin to Mn(II) hemoglobin.

The concentration of Mn(III) hemoglobin was determined as described by Waterman and Yonetani (16). Circular dichroic measurements were performed with a Jasco J-40 C automatic recording circular dichrograph using a cell path lenght of 1 mm. Raw CD curves and background curves were digitized; then backgrounds were subtracted out and averaged on an IBM 370/168. Final plots were made on a Calcomp 54" flat bed plotter (18).

RESULTS AND DISCUSSION

Titration of apohemoglobin with Mn(III) protoporphyrin IX revealed 1 mole of porphyrin bound per mole of hemoglobin subunit. The CD spectra of Mn(II)Hb and Mn(III)Hb in the 280-490 nm region are shown in Fig. 1. The general features of the 280 nm region are similar to that of deoxyHb(T) and aquometHb(R), respectively. The ellipticity in the 280 nm region is thought to arise from the aromatic moieties tyrosine C7(42)α and tryptophan C3(37)β(3). The stripped Mn(III) hemoglobin showed characteristic differences from the stripped aquomet Hb. The ellipticity of the stripped Mn(III)Hb in this region is very slightly negative in contrast to stripped aquometHbA which shows a weak positive ellipticity. These CD results can be interpreted as small changes in the environment of C7(42)α due to perturbations of threonine C3(38)α and C3(37)β due to perturbations of C5(39)β in the α_1-β_2 interface observed by Moffat *et al.* in the X-ray structure of Mn(III)Hb compared to aquometHb (14). These interchain contacts may, in turn, be reflections of the subtle difference in the metal environment between Mn(III)Hb and aquometHb. Addition of IHP does convert the stripped Mn(III)Hb spectrum in the 280 nm region to that characteristic of the T form (Fig. 2). This CD change in the 280 nm region upon the addition of IHP is much more like the change occurring with aquometHb (similar but not identical) rather than the invariance observed with cyanometHb or Co(III)Hb (3,7). That is, if the conformational changes are linked to metal environment changes Mn(III)Hb, aquometHb could be classified as flexible metal in environments and cyanometHb and Co(III)Hb could be classified as rigid metal in environments. It is interesting to note that the CD changes in the Soret region parallel the changes in the 280 nm region for the Mn(II)Hb, Mn(III)Hb as well as the effect of IHP on Mn(III)Hb.

The azide derivative of metHb, [N_3Fe(III)Hb], in the 280 nm region gives a CD spectrum characteristic of the R form and is invariant to IHP addition.

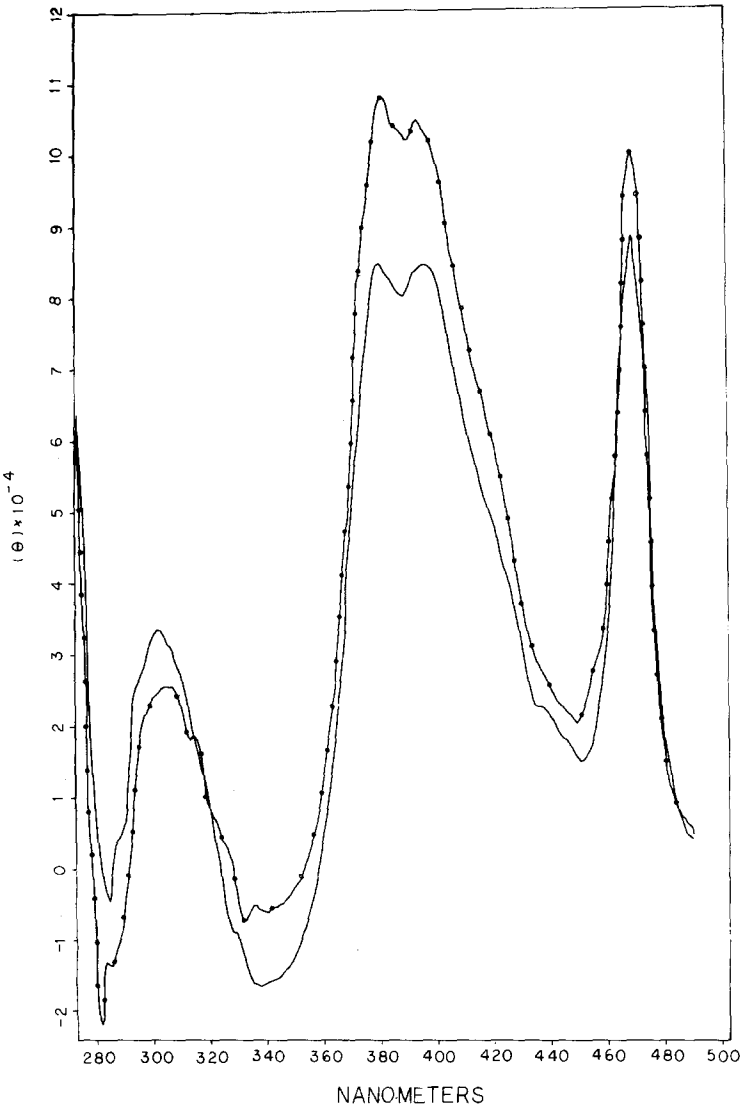

Figure 2: CD spectra of 58 μM (tetramer) solution of Mn(III) hemoglobin in the presence and absence of IHP buffered in 50 mM bis-TRIS of pH 6.5 (——) Mn(III) hemoglobin, (o-o-o-) Mn(III) hemoglobin + 4 moles IHP per mole of hemoglobin. Ellipticities are reported in terms of tetramers.

In contrast, N_3Mn(III)Hb, Fig. 3, shows a change in the 280-290 nm region relative to stripped Mn(III)Hb but the addition of IHP converts the spectrum to that characteristic of the T form. That is, the aromatic region of the uv CD spectrum of N_3Mn(III)Hb is similar to that of stripped Mn(III)Hb and the CD spectrum of N_3Mn(III)Hb + IHP is similar to that of Mn(III)Hb + IHP.

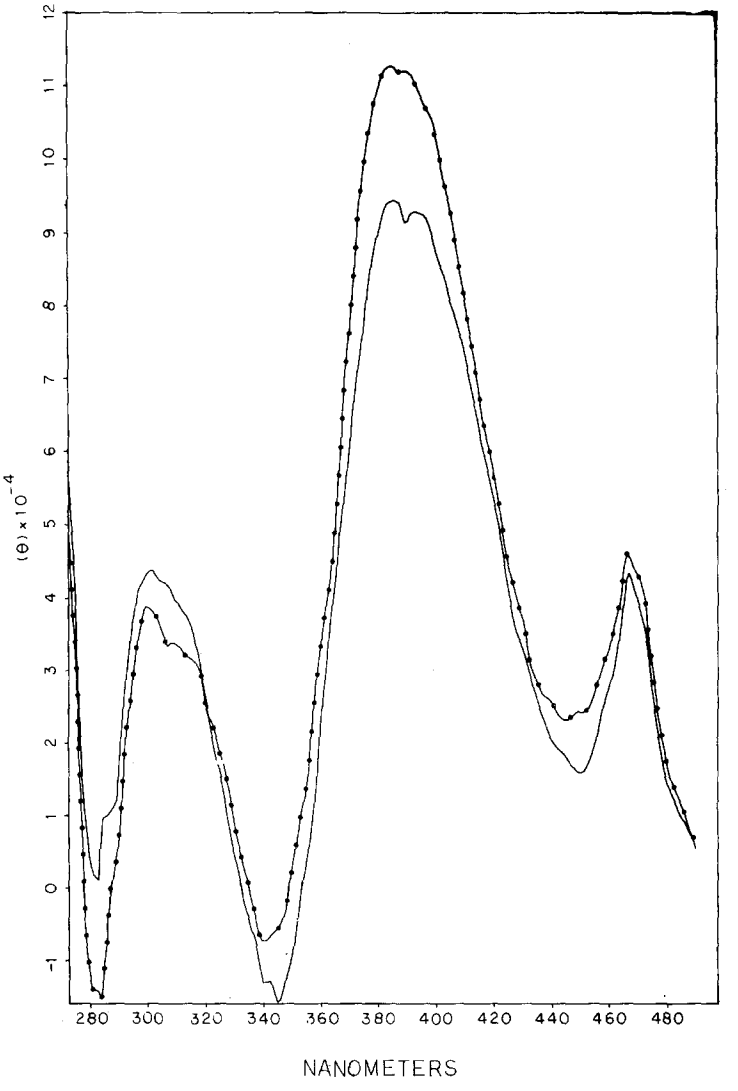

Figure 3: CD spectra of a 58 μM (tetramer) solution of azide Mn(III) hemoglobin in the presence and absence of IHP buffered in 50 mM bis-TRIS of pH 6.5. (——) azide Mn(III) hemoglobin, (o-o-o-o) azide Mn(III) hemoglobin +4 moles IHP per mole of hemoglobin. Ellipticities are reported in terms of tetramers. Azide concentration was 0.03 M.

However, such a statement is not applicable to the soret region in which the two CD bands decrease in intensity in the following order: N_3Mn(III)Hb > Mn(III)Hb > Mn(III)Hb + IHP. This behavior of the CD bands in the Soret region crudely parallels a decrease in the absorption intensity of these bands for the metalloporphyrin itself upon the addition of azide. We interpret these azide results in terms of weak Mn(III)-N_3 binding in contrast to

strong Fe(III)-N_3 azide binding in azide metHb. This is not to imply that the azide is readily dissociated from azide Mn(III)Hb but rather that both the Mn(III)F8 histidine linkage is weaker as well as the Mn(III)-N_3 linkage compared to the Fe(III) situation (19). Model studies of six coordinate azide LMn(III)porphyrin complexes shows that the Mn(III)-N (azide) bond is $\sim$.013A longer than in five coordinate Mn(III)-N(azide)porphyrins with Mn(III) slightly displaced from the heme plane toward the azide ion (10,11). This situation would make the metal ion position more flexible and allow movement of the metal ion when quaternary effector molecules such as IHP are introduced into the tetramer. In addition, the changes in the CD spectra in the Soret region with IHP for the azide Mn(III)Hb are consistent with this interpretation.

The 280 nm region CD results for the Mn(II)Hb to Mn(III)Hb oxidation indicate subtle quaternary structure differences between Mn(III)Hb and aquometHb. These results show that the 280 nm region CD spectra is a powerful and useful conformational tool for Hb's.

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