CIRCULAR DICHROISM OF HUMAN HEMOGLOBIN-HAPTOGLOBIN COMPLEXES

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Summary

The circular dichroism spectra (600 - 240 mμ) of four derivatives of human hemoglobin-haptoglobin complexes have been measured. The circular dichroism spectra of the human hemoglobin-haptoglobin complex are different from those of hemoglobin A and its subunits in the Soret region and in the 310 - 240 mμ region. The spectral difference in the Soret region between the hemoglobin-haptoglobin complex and hemoglobin A and its subunits indicates the presence of difference in the heme environment or a difference in the tightness of the heme attachment, or both, between the hemoglobin-haptoglobin complex and hemoglobin A and its subunits.

Haptoglobins (Hp) bind hemoglobin (Hb) stoichiometrically to form very stable complexes (Jayle et al. 1962). The β chain of Hp (Gordon et al. 1966) and the globin moiety of hemoglobin (Van Royen et al. 1950, Nyman 1959) are considered to be involved in the formation of the hemoglobin-haptoglobin complex (Hb-Hp complex) and electrostatic and hydrophobic bonds apparently participate in this formation (Robert et al. 1956).

The Hb-Hp complex has a high affinity for oxygen, no "heme-heme interaction", no Bohr effect (Nagel et al. 1965), broader and flatter absorption band at the Soret region of the deoxygenated derivatives (Chiancone et al. 1966), and a high combination rate of reaction with carbon monoxide (Ronald et al. 1966). These properties of the Hb-Hp complex resemble those of the isolated α and β subunits of Hb A, Hb H, and myoglobin.

In this communication, the circular dichroism (CD) of four derivatives of the human Hb-Hp complex is reported. The CD spectra of the Hb-Hp complex are different

from those of Hb A in the Soret region and in the 310 - 240 mμ region in every derivative examined. By comparison with the CD spectra of isolated α and β subunits of Hb A (Ueda et al. 1969), it is also shown that the CD spectra of the Hb-Hp complex in the Soret region are not the arithmetic means of the CD spectra of the separated chains. The spectral difference in the Soret region between the Hb-Hp complex and Hb A and its subunits seems to indicate that the difference in the heme environment or the difference in the tightness of the heme attachment, or both, in Hb A is caused by the binding of Hp.

Materials and methods

Three common phenotypes of oxygenated Hb-Hp complexes were purified as described previously (Hamaguchi 1968). Human adult oxygenated Hb was prepared by the toluene procedure (Drabkin 1942). Oxygenated derivatives of the Hb-Hp complex and Hb were dialyzed against 0.05 M phosphate buffer at pH 7.0. All three other derivatives of the Hb-Hp complex and Hb were prepared from the corresponding oxygenated derivatives by addition of a five-fold excess of potassium ferricyanide followed by exhaustive dialysis against 0.05 M phosphate buffer at pH 6.5 in the case of ferric derivatives, by saturation with CO gas in the case of carbomonoxy derivatives, and by the same arrangement described by Ueda et al. (Ueda et al. 1969) in the case of deoxygenated derivatives. The concentration of heme was determined by spectral analysis after conversion to pyridine hemochromogen. Human Hp of genetic type of 2 - 1 was prepared as described elsewhere (Hamaguchi 1969).

CD measurements were performed at room temperature on a Jouan dichrograph. The heme concentration of samples varied from 50 to 150 μM. The slit-width was maintained at less than 1.0 mm by controlling the voltage. Cells having a path length 1 mm, 5 mm and 10 mm were used. Molar ellipticities are given on a heme basis.

Results and discussion

Fig. 1 shows the CD spectra in the 460 - 240 mμ region of the oxygenated

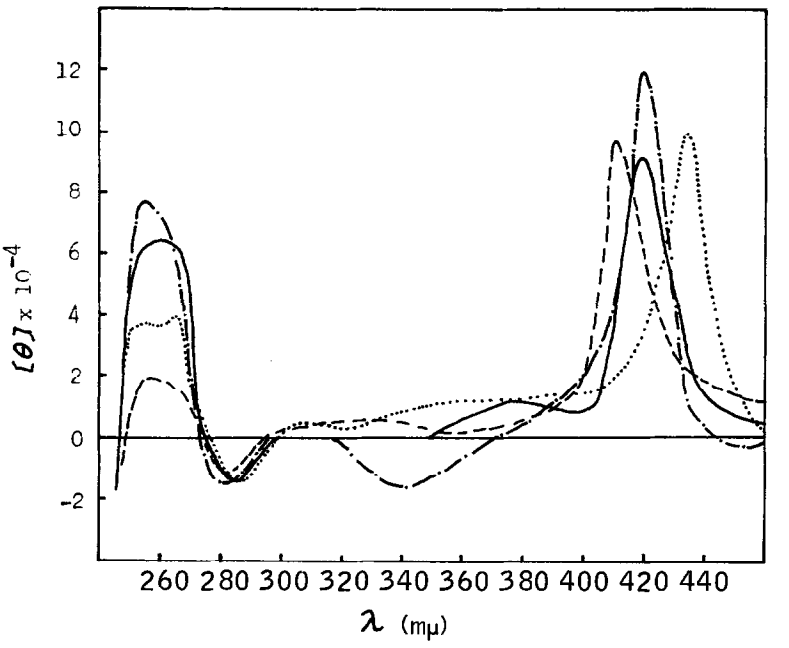

Fig. 1. Circular dichroism spectra of human Hb-Hp 2-2 complex between 240 and 460 mμ. Oxygenated derivative (————), carbomonoxy derivative (—— · —— · ——) and deoxygenated derivative (·····) were measured in 0.05 M phosphate buffer, pH 7.0. Ferric derivative (- - - - - -) was measured in 0.05 M phosphate buffer, pH 6.5.

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derivative, the ferric derivative, the carbomonoxy derivative and deoxygenated derivative of the human Hb-Hp complex. No significant difference in the CD spectra was observed among the three common phenotypes of Hb-Hp complexes. Every derivative represented shows a positive extremum at around 260 mμ and a negative trough at around 280 mμ. A prominent positive extremum in the Soret region is also shown in every derivative examined at around 420 mμ in the case of the oxygenated derivative, at around 412 mμ in the case of the ferric derivative, at around 420 mμ in the case of carbomonoxy derivative, and at around 435 mμ in the case of the deoxygenated derivative.

Fig. 2 shows the CD spectra in the 460 - 240 mμ region of liganded and unliganded derivatives of human adult Hb. Comparison of the spectra in Fig. 1 and 2

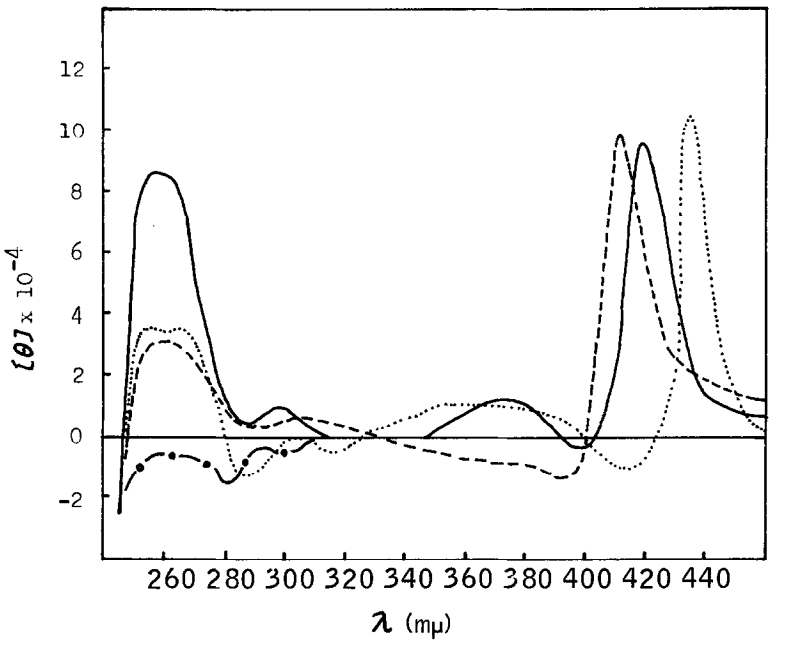

Fig. 2. Circular dichroism spectra of human adult Hb and Hp 2-1 between 240 and 460 mμ. Oxygenated derivative (————) and deoxygenated derivative (·····) of Hb and Hp 2-1 (—•—•—) were measured in 0.05 M phosphate buffer, pH 7.0. Ferric derivative (- - - - - - -) of Hb was measured in 0.05 M phosphate buffer, pH 6.5.

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shows that although the corresponding derivatives of the Hb-Hp complex and Hb has a similar magnitude of positive extremum at the same wavelength around 260 mμ and in the Soret region, the CD spectra of the Hb-Hp complex are different from those of adult Hb. A negative trough at around 280 mμ which is observed in every derivative of the Hb-Hp complex is absent in the CD spectra of the liganded derivative of Hb. In addition to the difference in the CD spectra in the 310 - 240 mμ region, a negative trough in the Soret region which is observed in every derivative of Hb disappears in every derivative of the Hb-Hp complex. Inspection of the CD spectra of the separated α and β chains of Hb A (Ueda et al. 1969) also shows that the CD spectra of the Hb-Hp complex in the Soret region are not the arithmetic means of the separated chains of Hb A, because a negative trough is absent in the Hb-Hp complex.

In Fig. 2 is also shown the CD spectra of human Hp. Molar ellipticity is given on a heme basis which is calculated from the Hb Binding Capacity of Hp. From 240 mμ to 310 mμ, the ellipticity of Hp is negative with a negative trough at around 280 mμ. In the region of wavelengths above 310 mμ, no ellipticity is observed.

These observations suggest that although the difference in the CD spectra in the 310 - 240 mμ region between the Hb-Hp complex and Hb is mainly due to the presence of Hp in the Hb-Hp complex, the spectral difference in the Soret region must reflect the difference in the heme environment or a difference in the tightness of the heme attachment, or both, between the Hb-Hp complex and Hb and its subunits. These differences may be related to the considerable increase in the peroxidase activity of Hb by the binding of Hp.

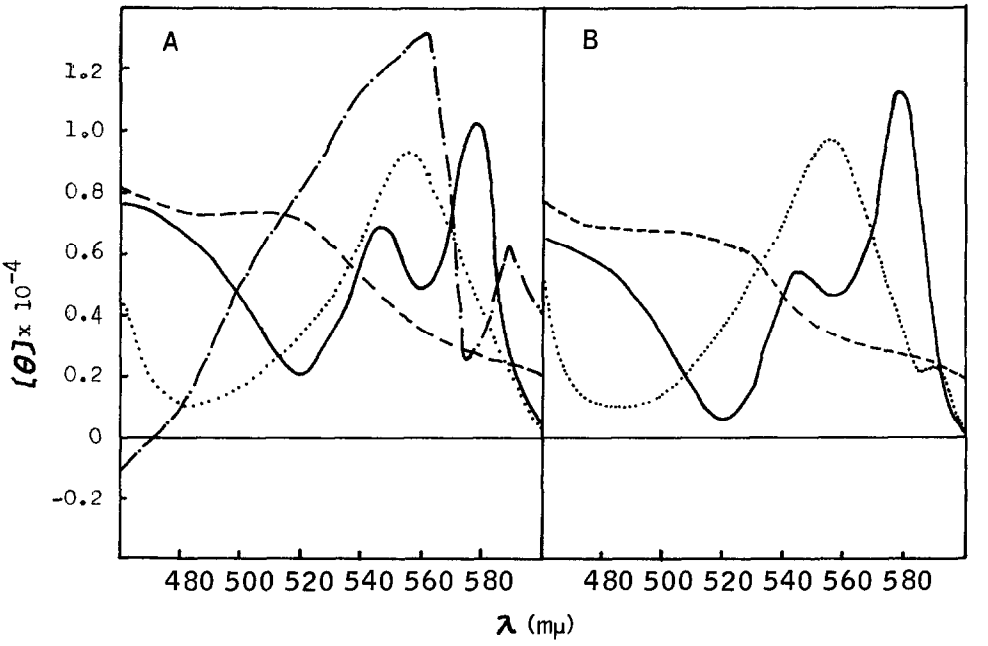

Fig. 3. Circular dichroism spectra of human Hb-Hp 2-2 complex and adult Hb between 460 and 600 mμ. A, Hb-Hp 2-2 complex ; B, adult Hb. Oxygenated derivative (————), carbomonoxy derivative (—— · —— · ——) and deoxygenated derivative (·····) were measured in 0.05 M phosphate buffer, pH 7.0. Ferric derivative (- - - - - -) was measured in 0.05 M phosphate buffer, pH 6.5.

Fig. 3 shows the CD spectra in the 460 - 600 mμ region, respectively, of liganded and unliganded derivatives of the Hb-Hp complex and human adult Hb. Comparison of the CD spectra in Fig. 3 shows that every derivative of the Hb-Hp complex examined has CD spectra similar to the corresponding derivative of the adult Hb, except that a shoulder at around 590 mμ of deoxygenated Hb is absent in the deoxygenated Hb-Hp complex. A similar phenomenon is observed in the CD spectra of the isolated subunits of Hb A (Ueda et al. 1969).

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