CIRCULAR DICHROISM SPECTRA OF HUMAN ADULT HEMOGLOBIN AND ITS SUBUNITS

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Summary

Studies of the circular dichroism (CD) of the α (Hb α) and β (Hb β) subunits of human adult hemoglobin (Hb A) have shown that the intensities of the principal bands in a spectral region from 215 to 610 mμ are much greater for Hb α than for Hb β and the intensities for Hb A are roughly the arithmetic means of the intensities of the isolated subunits.

The optical activity of a chromophore in a protein molecule reflects the local environmental structure of the chromophore. Thus the CD spectrum of hemoglobin gives us useful information not only on the analysis of its absorption spectrum but also on the estimation of its conformational changes induced by various means. The present report is an extension of a previous study (1), and includes visible spectral region and CO derivatives.

The hemoglobins were prepared by the methods described previously (2). Hb A solutions were further passed through a Sephadex G-25 column equilibrated with 0.1 M NaCl to remove organic phosphates (3), then adjusted to a desired pH. The isolated chains contained no detectable amount of the phosphates. The deoxygenation was carried out by stirring the hemoglobin solutions under nitrogen gas (purity 99.999%) stream. CD measurements were performed at 20° C on a Jouan dichrograph with 1 cm quartz cuvettes. Molar ellipticities (θ) are given on a heme basis. The half-intensity bandwiths for the spectral region of 450 ~ 610 mμ, 350 ~ 450 mμ, and 210 ~ 350 mμ were 6 ~ 10 mμ, 5 ~ 15 mμ, and 2 ~ 10 mμ, respectively.

In Fig. 1 are shown the visible CD spectra. Irrespective of the nature of the ligand attached to heme, Hb α shows more intense CD bands than Hb β,

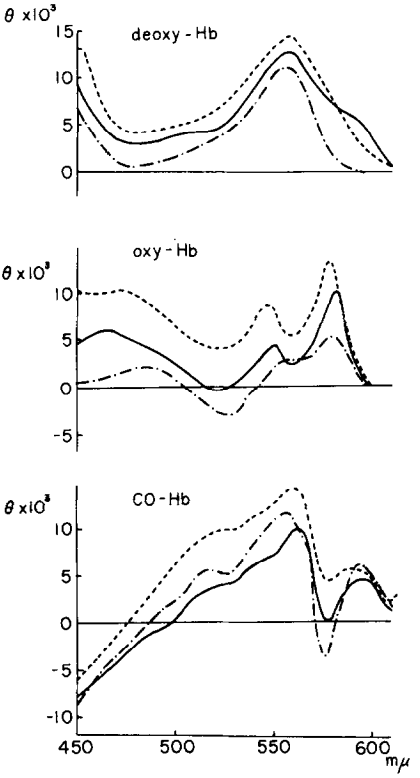

Fig. 1. CD spectra of hemoglobins in the visible region.
The concentration of hemoglobins (on a heme basis) is 1.6×10^{-4} M, in 0.05 M Tris buffer, pH 7.4

and the intensities of the bands for Hb A are roughly intermediate between those for the isolated subunits. Further, the following characteristic differences can be noticed among the spectra for three kinds of hemoglobins. In the deoxy form Hb α and Hb A exhibit a band at around 580 mμ, which is hardly observed in the spectrum for Hb β. In the oxy derivative, a CD band near 525 mμ is positive for Hb α whereas it is negative for both Hb A and Hb β. A negative band in the 575 mμ region observed in the CO form is less apparent in Hb α than in Hb β.

The Soret CD spectra are shown in Fig. 2. Again, the CD bands for Hb α are more intense than those for Hb β and the bands for Hb A are intermediate intensity. In the deoxy form, Hb α shows a positive broad band near 470

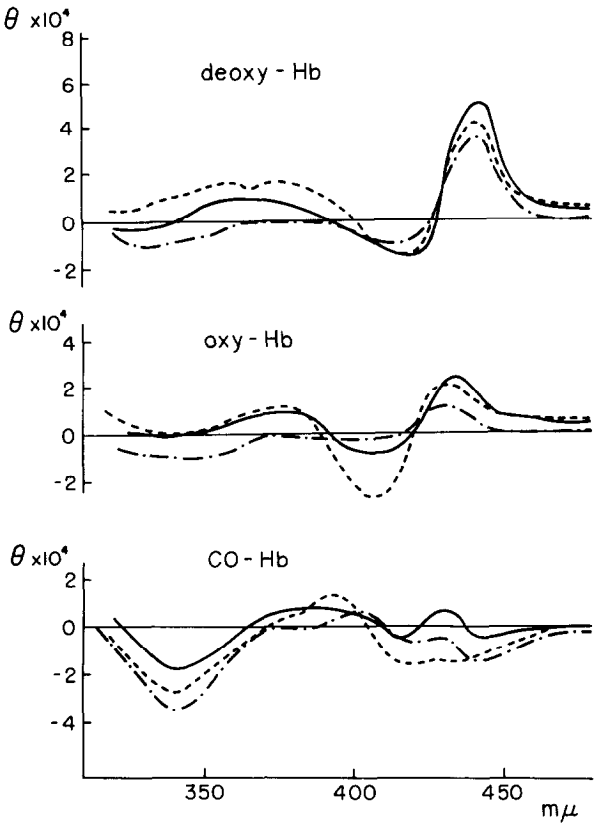

Fig. 2. CD spectra of hemoglobins in the Soret region.
The concentration of hemoglobins (on a heme basis) is 5×10^{-5} M, in 0.05 M Tris buffer, pH 7.4.

and 380 mμ, whereas no such bands are observed in Hb β. A band at around 350 mμ is positive for deoxy Hb α but negative for deoxy Hb β. A distinct negative band near 405 mμ seen in oxy Hb α is absent in oxy Hb β. In the CO derivative, the differences in the CD peaks of the subunits become evident and the spectrum for Hb A displays a complicated pattern. The spectral characteristics of the Soret CD band depend upon the slit width, and the spectra shown in Fig. 2 are valid only at the half-intensity bandwidths employed. On narrowing the slit width, the positive peaks shift to the shorter wavelength side with an increase in θ values and the intensity of the negative peaks decrease. This clearly indicates that the Soret CD bands consist of positive and negative bands and will explain quantitative differences

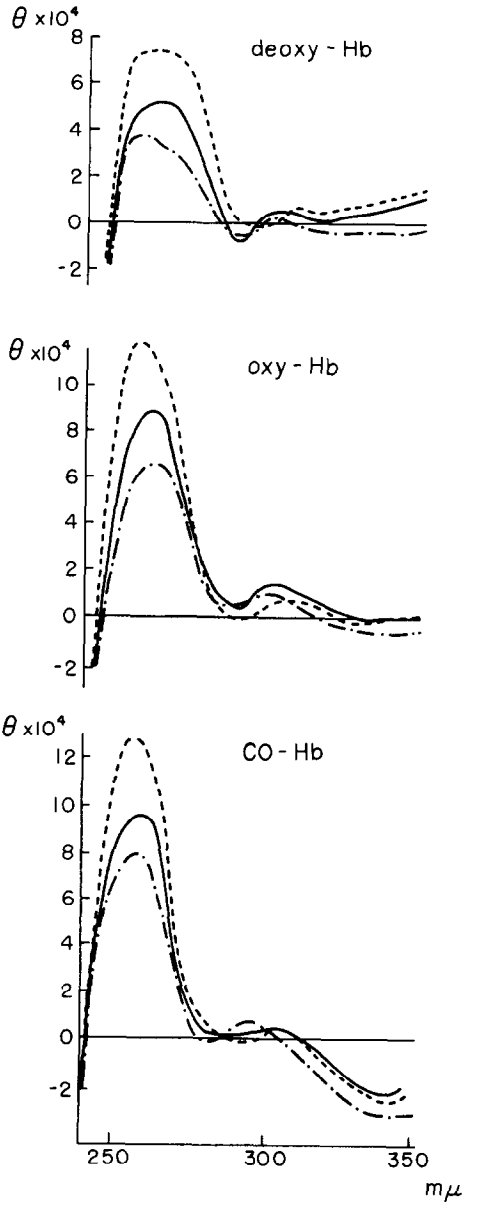

Fig. 3. CD spectra of hemoglobins in the UV region.
The concentration of hemoglobins (on a heme basis) is 5×10^{-5} M, in 0.05 M Tris buffer, pH 7.4.

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between the spectra shown in Fig. 2 and those reported by Sugita et al. (4).

Fig. 3 depicts the CD spectra in the ultraviolet region. A distinct difference can be seen between the subunits in peak position of the band near

300 mμ, which is responsible for the broad and asymmetric band of Hb A in this region. The 260 mμ band can be resolved into at least two positive bands, especially in the deoxy form. A weak negative band observed in deoxy Hb A at 280 mμ region is also seen in deoxy Hb β but in a lesser intensity.

The CD spectra in 220 mμ region were also studied. However, no significant difference was observed among the three hemoglobins in the three forms. Helix content calculated from the ellipticity at 222 mμ range from 65 to 75% in all the samples.

The distinction of the CD spectra of Hb α and Hb β described above clearly indicates the difference in the local field, i.e. the environmental structure, of protoheme of the two subunits, which may have some connection with the slight difference in oxygen affinity of the subunits (1). The results of our recent studies on the subunits by electron paramagnetic resonance (5) and difference absorption spectrophotometry (6) also support the view. The CD spectrum of Hb A can be considered, in first approximation, as the arithmetic composite of the spectra of the two subunits. Thus, the environmental structure around the hemes of α and β chains seems to be preserved upon recombination yielding the $\alpha_2\beta_2$ tetramer in all the derivatives studied.

Resolution of the CD and absorption spectra of the hemoglobins into their components are now in progress.

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