CIRCULAR DICHROISM OF LAMPREY AND HUMAN HEMOGLOBINS

Yoshiki Sugita, Yoshiko Dohi and Yoshimasa Yoneyama

Department of Biochemistry, School of Medicine

Kanazawa University, Kanazawa, Japan

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The optical rotatory dispersion (ORD) of hemoglobin and myoglobin has been described over the region from 220 to 700 mμ (Beychok and Blout, 1961, and Samejima and Yang, 1964). Though circular dichroism (CD) is a more useful technique than ORD for distinguishing optically active transitions, the CD spectra of hemoglobin and myoglobin have recently reported only in the ultraviolet region (Urry, 1967, and Beychok et al, 1967). Lamprey hemoglobin shows heme-heme interaction similar to other vertebrate hemoglobins, and yet it is a monomer when oxygenated and aggregates on deoxygenation (Briehl, 1963). In this communication we report the CD of lamprey hemoglobin and human hemoglobin. The CD spectrum of lamprey hemoglobin was different from that of human hemoglobin in the Soret region, in the 300-240 mμ region and below 240 mμ.

Lamprey blood was obtained from Entosphenus japonicus by heart puncture. Two hemoglobin components were separated on sucrose density gradient electrophoresis at pH 8.6 and the major component was used in the experiment. The CD spectra were recorded at 20° on a JASCO ORD/UV5 spectropolarimeter with a CD attachment. Deoxygenated hemoglobin was prepared by evacuation in Thunberg type cells having path lengths 1 cm and 0.1 cm. When the cell with path

length 0.01 cm was used, the deoxygenation was carried out in a controlled atmosphere glove bag. In some occasions a few grains of dithionite were added for reduction. The addition of dithionite gave no effect on the CD curve in the visible region. CD spectra were run immediately after the deoxygenation. Then the samples were allowed to be oxygenized by air and the spectra were run again. Concentrations of hemoglobins were determined by spectral analysis after conversion to pyridine hemochromogens.

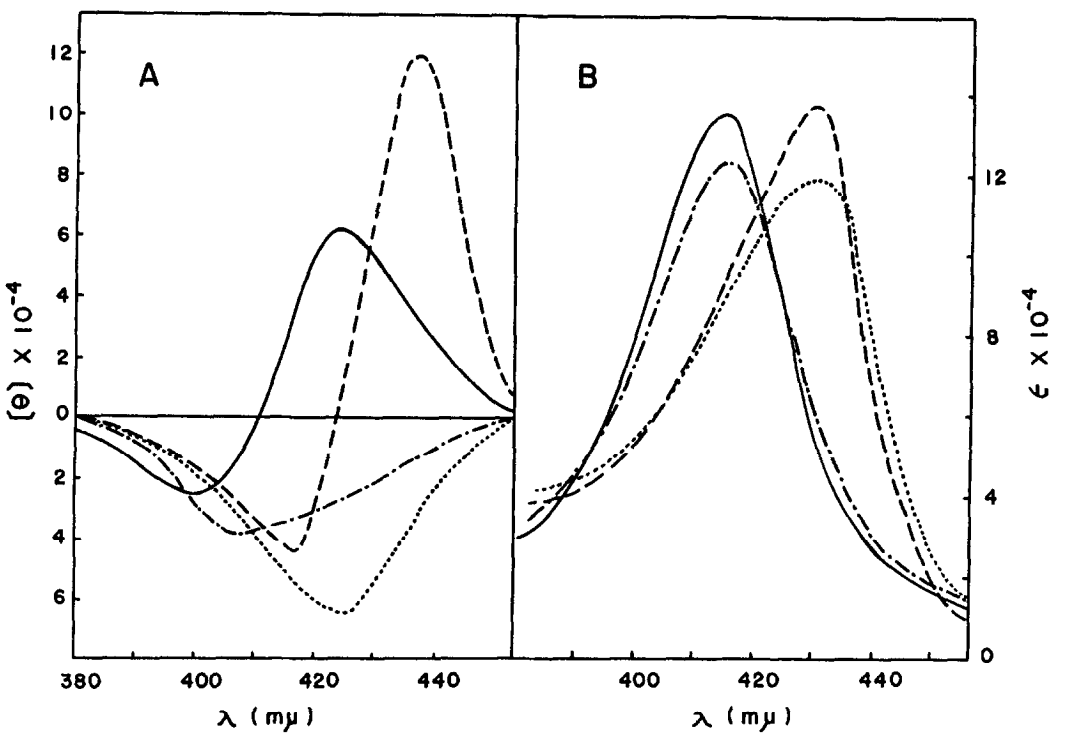

Fig. 1 Circular dichroism and absorption spectra of human and lamprey hemoglobins in the Soret region. The heme concentrations were 200 μM in 0.1 M phosphate buffer, pH 7.0. The ellipticities (θ) are on a molar heme basis. ————, oxygenated human Hb; --------, deoxygenated human Hb; —·—·—·—, oxygenated lamprey Hb; and ....., deoxygenated lamprey Hb.

Fig. 1A and 1B show the CD spectra and absorption spectra in the Soret region, respectively, of oxygenated and deoxygenated human and lamprey hemo-

globins. Oxygenated human hemoglobin exhibits complex CD with a positive extremum at 423 mμ and a negative trough at 403 mμ. Oxygenated lamprey hemoglobin possesses in this region only a negative peak at about 408 mμ with a shoulder at about 425 mμ. When hemoglobins were deoxygenated, the positions of the peaks in CD shifted to longer wave lengths and absolute ellipticities increased in both lamprey and human hemoglobins. These shifts of the peaks correspond to those in absorption spectra.

Inspection of the CD and absorption spectra of oxygenated human hemoglobin suggests that a minimum of two Gaussian functions, centered at about 415 mμ and at about 410 mμ, would be required to approximate the data. We do not wish to argue that the resolution into a set of curves are unique but the results are interesting. The CD and absorption spectra of oxygenated lamprey hemoglobin require a minimum of three Gaussians at about 405-410 mμ, 417 mμ and 425 mμ. The resolved peak at 417 mμ in CD which fits the peak in the absorption spectrum is very small compared to the corresponding peak in human hemoglobin. Zand and Vinogradov (1967) observed very different CD spectra in the Soret region between horse heart ferricytochrom c and Pseudomonas aeruginosa ferricytochrome c. The spectra were described as approximate mirror images of one another, suggesting the different arrangemant of ligands in positions 5 and 6 of the heme group. The difference between the CD curves of the two cytochromes closely resembles to that between the CD of lamprey and human hemoglobins. Amino acid analysis of the hemoglobin from Entosphenus japonicus showed that it contained two histidine per molecule and similar amino acid composition to that of Lampetra fluvitalis reported by Rudloff et al (1966). As a result of the sequential analysis of lamprey hemoglobin, Rudloff et al showed that the positions of two histidine coincided with those in human hemoglobin which coordinate to the iron of heme. These findings suggest that the difference between the CD spectra of the two hemoglobins is probably due to the difference in the heme environment caused by the tertiary structure, rather than to the different coordination groups to heme. The

complex CD curve of human hemoglobin bears striking resemblance to that of ferrocytochrome oxidase which was reported to arise from exciton resonance interaction of the transition in juxtaposed heme (Urry et al, 1967), although the closest iron atoms in human hemoglobin were 25 Å apart and the nearest hemes are aligned nearly head to tail.

Fig. 2 shows the CD spectra in the ultraviolet region from 300 to 240 mμ. Oxygenated and deoxygenated lamprey hemoglobin exhibit almost identical negative bands between 300 and 270 mμ, whereas human deoxygenated hemoglobin shows negative bands and oxygenated form shows no appreciable band in this region.

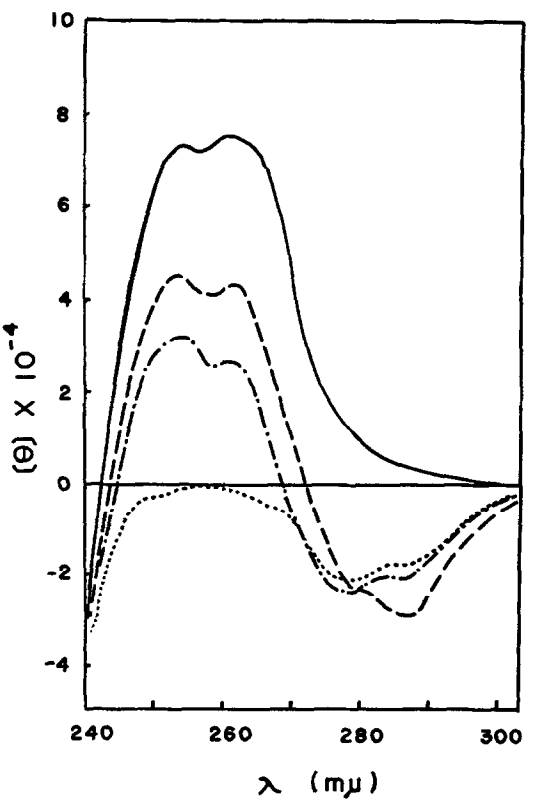

Fig. 2 Circular dichroism of human and lamprey hemoglobins. The ellipticities are on a molar heme basis. Heme concentrations were 200 μM in 0.1 M phosphate buffer, pH 7.0. ————, oxygenated human Hb; – – – – –, deoxygenated human Hb; —·—·—·—·—, oxygenated lamprey Hb; and ....., deoxygenated lamprey Hb.

Between 270 to 240 mμ human hemoglobin exhibits positive band and the ellipticity increases on oxygenation. Oxygenated lamprey hemoglobin possesses positive band at about 260 mμ but deoxygenated derivative showed no positive value. The positive band at about 260 mμ was ascribed to optically active heme transitions by Urry (1967) through the work with ferriheme undecapeptide which contained no aromatic amino acid. The difference in CD at 260 mμ between lamprey and human hemoglobins, in addition to that in the Soret region, suggests that two hemoglobins have different heme environments.

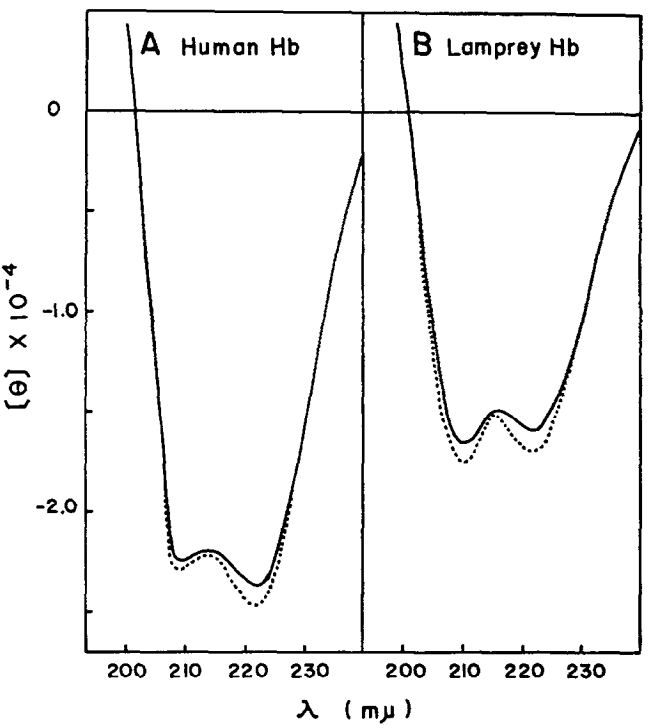

Fig. 3 Circular dichroism between 200 and 240 mμ. A, human hemoglobin; B, lamprey hemoglobin. ————, oxygenated hemoglobins; ....., deoxygenated hemoglobins. The heme concentrations were 200 μM in 0.1 M phosphate buffer, pH 7.0. The ellipticities are given on a mean residue basis using 143.5 and 156, respectively, for the numbers of amino acid residues per heme of human and lamprey hemoglobins.

Below 240 to 200 mμ, the ellipticities of both human and lamprey hemo-

globins become negative as shown in Fig. 3. Lamprey deoxygenated hemoglobin exhibits negative extrema at 222 mμ and 209 mμ with about 10 per cent larger magnitude than those of oxygenated hemoglobin in the conditions described in Fig. 3. This increase in negative ellipticity on deoxygenation is similar to that found in human hemoglobin. The CD band at 222 mμ are considered to be characteristic of α-helix. The negative ellipticity of lamprey hemoglobin at 222 mμ had the magnitude nearly two thirds that of human hemoglobin. This smaller band at 222 mμ suggests that lamprey hemoglobin molecule contains less α-helix than human hemoglobin. The effect of aggregation of lamprey hemoglobin on CD and absorption spectra is under investigation.

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