LIGAND-INDUCED REVERSAL OF THE SORET CD BAND OF LAMPREY HEMOGLOBIN

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1. Introduction

Studies on circular dichroism (CD) of hemoproteins in the Soret region provide information about the molecular structure of the heme environment [1, 2]. In general hemoglobins show positive CD bands in the Soret region. A negative CD band is described only for Chironomus hemoglobin with trivalent heme iron [3] and for lamprey hemoglobin in the deoxy and oxygenated form [4]. Investigations of further complexes of this hemoglobin show that the negative Soret CD band is converted to intense positive CD bands, especially by binding of alkyl isocyanides. This effect was analysed with respect to the ligands, because the reversal obviously was effected only by ligands which, besides being bound to the heme iron, interact with the protein of the heme environment.

2. Material and methods

Hemoglobin was prepared from the blood of freshly caught lamprey (*Lampetra fluviatilis*) as described elsewhere [5, 6]. The alkyl isocyanides were obtained using the method described by Gautier [7], and distilled twice. Circular dichroism was measured with a CD 185 dichrograph from the Jouan Co. The dichrograph was calibrated with epiandrosterone and programmed to constant energy so that the slit width varied from 0.5 to 2.0 mm. Concentration and thickness of layers of the cuvettes were selected in such a way as not to overload the device.

3. Results

Figs. 1 and 2 show CD spectra of deoxy-Hb, oxy-Hb, NO–Hb, CO–Hb, nitrosobenzene–Hb and of the alkyl isocyanide complexes investigated. In the spectral region between 240 and 500 nm all hemoglobin derivatives, irrespective of the sign of ellipticity, show two characteristic CD bands: at 260 nm and in the Soret region at about 420 nm. In the Soret region deoxy-Hb shows a negative CD band at 428 nm. Whereas by oxygenation a reduction and a blue shift of the Soret CD band is caused, the binding of NO induces an increase of the negative CD band. On the other hand, nitrosobenzene produces a reversal of the Soret CD band. Fig. 1 shows that by nitrosobenzene binding a positive CD band with lower intensity is generated than by the binding of alkyl isocyanides. An investigation of the nitrosobenzene–Hb complex below 340 nm was impossible because of the high signal-to-noise ratio. CO–Hb has an intermediate position between NO–Hb and oxy-Hb on the one hand and nitrosobenzene–Hb and alkyl isocyanide complexes on the other. It shows a negative CD band in the longwave spectral region of the Soret band which changes into a positive one in the short-wave spectral region. The binding of alkyl isocyanides produced intense positive CD bands in the Soret region. The ellipticities of these bands are different, depending on the ligand. The intensity of ellipticity increases with the magnitude of the hydrophobic alkyl residue of the isocyanides (fig. 2). This relation is not detectable in the 260 nm region, in contrast to other vertebrate

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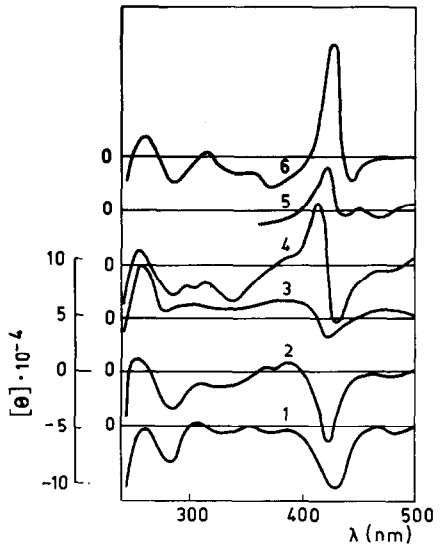

Fig. 1. Circular dichroic spectra between 240 and 500 nm of lamprey hemoglobin: 1) deoxy-Hb; 2) NO–Hb; 3) oxy-Hb; 4) CO–Hb; 5) nitrosobenzene–Hb; 6) ethyl isocyanide–Hb; 50 mM phosphate buffer, pH 6.7; hemoglobin concentration 1.2×10^{-3} M; temperature 22°; optical path 0.1 cm; hemoglobin was reduced with sodium dithionite.

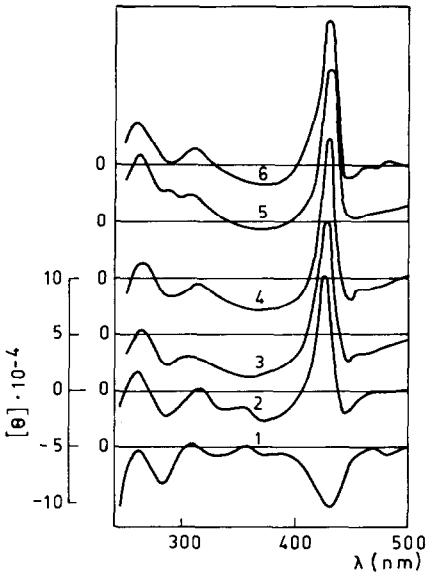

Fig. 2. Circular dichroic spectra between 240 and 500 nm of alkyl isocyanide complexes of lamprey hemoglobin: 1) deoxy-Hb; 2) ethyl isocyanide–Hb; 3) n-propyl isocyanide–Hb; 4) isopropylisocyanide–Hb; 5) n-butyl isocyanide–Hb; 6) tert.-butyl isocyanide–Hb; 50 mM phosphate buffer, pH 6.7; hemoglobin concentration 1.2×10^{-3} M; temperature 22°; optical path 0.1 cm; hemoglobin was reduced with sodium dithionite.

hemoglobins [1]. Fig. 1 shows that all ligands convert the negative absorption in the 260 nm region of deoxy-Hb into a positive one. The magnitude of this conversion depends on the nature of the ligand.

4. Discussion

The strength of optical activity in the Soret region is chiefly due to allowed π–π^* transitions in aromatic side chains of globin with the Soret transition of the heme chromophore [8]. The two components of the Soret transition of the heme plane B_x and B_y have opposite signs for their optical activity. The reversal of the CD band in the Soret region of lamprey hemoglobin by certain ligands means that the determining part of optical activity is converted from the B_y component (–) into the B_x component (+). This conversion of optical activity from B_y to B_x component can be explained by displacement of the heme chromophore. Obviously, the binding of nitrosobenzene and alkyl isocyanides distorts the heme disc in the heme pocket, inducing a new geometric position. For these ligands it is characteristic that, apart from their iron binding they can interact with the protein. With NO no such interaction is possible and therefore its binding fails to cause a reversal effect, in contrast to nitrosobenzene which, by virtue of its benzene ring, is able to interact with the protein.

On the reaction of oxygen or CO with the polymeric deoxy lamprey hemoglobin, the protein depolymerizes to the monomeric form [9–11]. As oxygenation is not connected with the reversal of the Soret CD band, the state of aggregation does not cause this observed effect. The question, if alkyl isocyanides bound to lamprey hemoglobin bring about also a depolymerization of the protein is under investigation (J. Behlke, personal communication).

In case of lamprey hemoglobin it is an interesting question, why a heme–heme interaction appears. Sigmoidicity in the O_2 and CO binding reaction

with lamprey hemoglobin was observed [9, 10, 12]. At the binding too, of alkyl isocyanides to lamprey hemoglobin, the *n* value ranged between 1.3 to 1.5 (J. Lampe, unpublished). Whatever is the cause for heme–heme interaction in lamprey hemoglobin, there is no correlation between the reversal of the Soret CD band effected by certain ligands and the heme–heme interaction, as sigmoidicity is observed in the oxygen binding as well as in the alkyl isocyanide binding reaction, but the reversal is effected only by the bond of alkyl isocyanides with lamprey hemoglobin.

The increase in positive ellipticity of the Soret CD band with increasing hydrophobic residue of the alkyl isocyanide (see fig. 2) means that an increased ligand–protein interaction also effects a stronger displacement of the heme disc or enforces such a displacement. Lamprey hemoglobin has, in contrast to other vertebrate hemoglobins, a particularly apolar heme environment [5, 13]. This hemoglobin therefore offers the possibility of intense interactions between the alkyl residue of the ligand and the protein. We suppose that in lamprey hemoglobin certain ligands interact intensively with apolar groups of the heme pocket and that this is the cause of the ligand-induced reversal effect.

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