ULTRAVIOLET CIRCULAR DICHROISM OF NITROSYL HEMOGLOBIN

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Received 24 September 1974

1. Introduction

Simon and Cantor [1] first demonstrated that the deoxygenation of hemoglobin produced a negative peak in the ultraviolet circular dichroism (CD) spectrum around 285 nm. Recently Perutz et al. [2,3] have shown that this negative CD peak indicates the R to T transition in hemoglobin independent of the degree of ligation. For hemoglobins which crystalize in a structure isomorphous with oxyhemoglobin this negative CD peak is not produced even in the absence of heme ligand. As Perutz et al. [2,3] have shown, this negative CD peak is due mainly to the aromatic amino acids at the $\alpha_1\beta_2$ contact region, in particular tryptophan C3 (37) β [2].

In this communication I report CD studies on adult nitrosyl hemoglobin which shows that the addition of inositol hexaphosphate (IHP) produces a large negative peak in the CD at about 285 nm whereas addition of IHP to the carbon monoxide form of adult hemoglobin does not show this effect. Furthermore, stripped adult nitrosyl hemoglobin does not show this negative CD peak. Based on the conclusions of Simon and Cantor [1] and Perutz et al. [2,3], the present results suggest that IHP switches fully saturated adult nitrosyl hemoglobin from the R to the T state.

2. Materials and methods

Isolated human adult hemoglobin was prepared as described elsewhere [4]. The NO bound form was prepared by first converting stripped oxyhemoglobin to the CO form and then to the NO form anaerobically. Gases used were from Matheson Inc. (East Rutherford, N. J.). IHP was prepared as described elsewhere [4] and was added to the protein from a stock solution in the same buffer to give a final concentration of 1.5 mM. The buffer used was 0.2 M Bis–Tris, pH 7.0. The CD spectra were measured on a Cary 61 spectrometer in 1 cm cells.

3. Results and discussion

Fig. 1, shows the ultraviolet CD for stripped nitrosyl and oxyhemoglobin. These spectra show weak positive ellipticity with two dips near 285 and 290 nm characteristic of the R state [2,3]. Fig. 2, shows the spectra for the CO and NO forms of hemoglobin with IHP present. The spectra for the CO form is like other R state CD spectra in that no large negative peaks are seen [2,3]. The nitrosyl form of adult hemoglobin + IHP. on the other hand, shows a large and rather broad negative peak with a minimum at about 285 nm, exactly the same as was observed in Perutz et al. [2,3] for other T state hemoglobins such as aquomet hemoglobin + IHP.

The idea that the fully liganded form of hemoglobin can take the deoxy quaternary structure is a consequence of the model of Monod, Wyman and Changeux [5]. The results presented here demonstrate that the fully liganded form of a low spin hemoglobin can take the deoxy conformation in agreement with the Monod model [5]. Recent high resolution nuclear magnetic resonance (NMR) studies [6] have shown that addition of IHP to adult nitrosyl hemoglobin in H_2O causes the -14 ppm line in the NMR spectra to appear. This line

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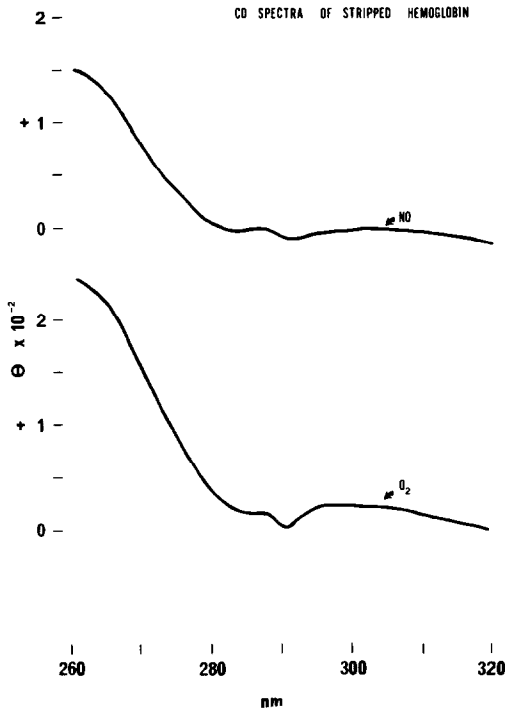

Fig. 1. Ultraviolet CD spectra of stripped adult nitrosyl (top) and oxy (bottom) hemoglobin in 0.2 M Bis–Tris, pH 7.0, 25°C. Concentration in heme was 24 μM for the nitrosyl form and 29 μM for the oxy form. The spectra are presented as ellipticity in degrees.

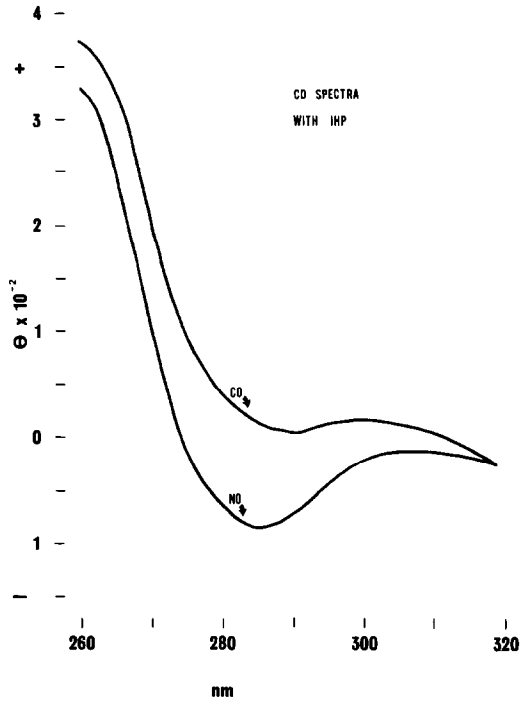

Fig. 2. Ultraviolet CD spectra of adult hemoglobin in the presence of IHP. Top spectrum is for the CO form and the bottom spectrum is for the NO form in 0.2 M Bis–Tris, pH 7.0, 25°C. Concentrations of heme and IHP were 53 μM and 1.5 mM respectively for both liganded forms. The spectra are presented as ellipticity in degrees.

is also indicative of the presence of the T state as discussed elsewhere [7].

The change in the spin state of the heme irons from low spin to high spin has been implicated in the structural transition of deoxy and met hemoglobins. However, the present results show that the same quaternary transition can occur in the absence of a change in spin state. However, from various electron paramagnetic resonance (EPR) studies on nitrosyl hemoglobin [8–10] it is conceivable that the unpaired electron of the NO molecule may be involved in the ability of nitrosyl hemoglobin to switch structure. The EPR spectra seen upon addition of IHP to nitrosyl hemoglobin [9] may indicate that a redistribution of the unpaired electron occurs within the imidazole–iron–NO complexes such that the bond length between the base molecule and the iron are affected as suggested from the studies of Kon and Kataoka [8]. The precise mechanism involved in the R → T transition of nitrosyl hemoglobin demonstrated by these CD results awaits further study.

Acknowledgements

I thank Drs R. G. Shulman and S. Ogawa for helpful discussions. I would also like to thank Professor Robert Haselkorn and Professor Paul Sigler for their support during my combined program of study between the University of Chicago and Bell Laboratories.

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