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# INTERACTION BETWEEN α1 AND β1 SUBUNITS OF HUMAN HEMOGLOBIN

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We prepared normal and modified α and β globin chains in which C-terminal residues were enzymatically removed. The CD spectra of the deoxy form of these chains and the reconstituted modified Hb's were measured in the Soret region. The CD spectra of the modified Hb's were markedly different from the arithmetic means of respective spectra of their constituent chains. This difference was ascribed to the interaction between α1 and β1 subunits to make the α1β1 dimer. The peak wavelength of the difference CD spectra could be classified into two groups, one was 433±1 nm and the other 437±1 nm. A comparison of this classification with the previously identified quaternary structures revealed that the R and T structures showed a maximum of the difference CD spectra at 437±1 nm and 433±1 nm, respectively. These results indicated that the R and T structures differed in the interaction between α1 and β1 subunits.

Hemoglobin ( Hb ) is a tetrameric protein composed of two α and two β chains. The quaternary structure of deoxy Hb is known to be different from that of oxy Hb [1,2]: the former is a so-called "T structure", the latter is a "R structure". In both the structures, α1β1 dimer has a very similar conformation and the intersubunit contacts at the α1β1 interface are identical [1-3]. Therefore, it has been generally accepted that the interaction between α1 and β1 subunits ( the α1 β1 interaction ) did not change during the R-T transition of quaternary structure.

The C-terminal residues of deoxy Hb form salt bridges and hydrogen bonds with specific residues [1]. These interactions help to stabilize

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Abbreviations: des-, modified by proteolytic removal of C-terminal residue(s) from α and/or β chains; IHP, inositol hexaphosphate; Tris, tris(hydroxymethyl)aminomethane; Bis-Tris, bis(2-hydroxyethyl)imino-tris(hydroxymethyl)methane.

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the deoxy Hb quaternary structure. Chemically modified Hb's, in which C-terminal residues are removed by carboxypeptidases A and B, are capable of undergoing the R-T structural interconversion within the deoxy state [1,4-10]. On the other hand, the CD spectra in the Soret resion are known to be changed upon assembling of isolated α and β chains to reconstitute Hb molecule [11]. The Soret CD change reflects the interaction between α1 and β1 subunits to make the α1β1 dimer from α and β chains [12-15].

In order to clarify whether the α1β1 interaction is really identical in the R and T quaternary structures, we investigated the Soret CD change upon assembling of the modified α and β chains to reconstitute the modified Hb's. The results indicate that the R and T structures differ in the α1β1 interaction.

## MATERIALS AND METHODS

Human Hb and its isolated α and β chains were prepared as previouly reported [12]. The modified chains were prepared from the isolated chains in the CO form by carboxypeptidases A and B ( Cooper Biomedical Inc., New Jersey ) as described by Kilmartin [16]. The digestion was monitored with a PICO-TAG amino acid analysis system ( Waters Ltd., Massachusetts ). Carbon monoxide was removed from the modified chains in a rotary evaporator under a stream of oxygen and strong illumination. Deoxygenation was achieved by addition of minimal amounts of sodium dithionite. Reconstitution was performed by mixing of equimolar concentrations of the α and β chains. All the protein concentrations were expressed on a heme basis, which was determined by the pyridine hemochromogen method. CD spectra and stopped-flow traces were measured at 15°C in a Union Giken Dichrograph III-J coupled with a NEC PC-9801F computer. The path of the observation cell was 10 mm.

## RESULTS AND DISCUSSION

CD Spectra in the Soret Region The Soret CD spectra of normal and modified α and β chains and those of reconstituted Hb's were measured under three conditions : pH 6.0 ± 1mM IHP or pH 9.0. The difference CD between the spectra of the reconstituted Hb's and the arithmetic means of the spectra of the corresponding constituent chains was obtained.

Figure 1A shows the Soret CD spectra of isolated α ( spectra a ), β chains ( spectra b ), des-Argα141 α chain ( spectra c ) and des-Hisβ 146 β chain ( spectra d ) in the presence and absence of IHP at pH 6.0 or at pH 9.0. The intensity of the CD band was affected by modification of the chain or buffer conditions, while the peak position of the CD band was not.

Figure 1B shows the Soret CD spectra of reconstituted normal Hb ( spectra e ) and reconstituted des-Argα141-des-Hisβ146 Hb ( spectra

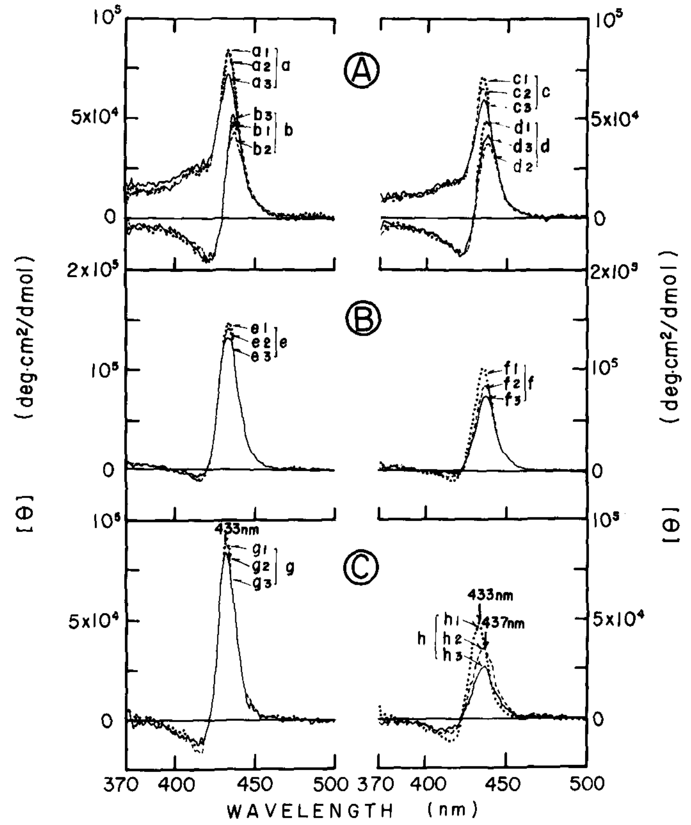


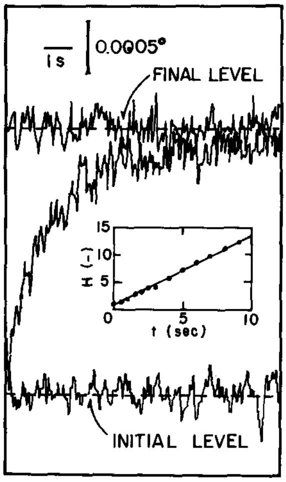
Figure 1. Soret CD spectra of chemically modified hemoglobins under three conditions : 0.1 M Bis-Tris, 0.1 M $Cl^-$ at pH 6.0 in the presence (----; a1,b1,c1,d1,e1,f1,g1 and h1 ) and absence (– – ; a2,b2,c2,d2,e2,f2, g2 and h2 ) of 1 mM IHP ; 0.1 M Tris, 0.1 M $Cl^-$ at pH 9.0 (——; a3,b3,c3, d3,e3,f3,g3 and h3). The ellipticity was expressed in molar ellipticity on a heme basis.
A; α chain (spectra a), β chain (spectra b), des-Argα141 α chain (spectra c), and des-Hisβ146 β chain (spectra d). B; Reconstituted normal Hb (spectra e), and reconstituted des-Argα141-des-Hisβ146 Hb (spectra f). C; The difference between the CD spectra of normal Hb and the arithmetic means of respective spectra of α and β chains (spectra g ; g1=e1-(a1+b1)/2, g2=e2-(a2+b2)/2, and g3=e3-(a3+b3)/2), and the difference between the CD spectra of reconstituted des-Argα141-des-Hisβ 146 Hb and the arithmetic means of respective spectra of their constituent chains (spectra h ; h1=f1-(c1+d1)/2, h2=f2-(c2+d2)/2, and h3=f3-(c3+d3)/2).

f ), which are equimolar mixtures of normal α and β chains for the former and of des-Argα141 α chain and des-Hisβ146 β chain for the latter. The intensity of the CD band of des-Argα141-desHisβ146 Hb was much smaller than that of normal Hb. The peak position of the CD band for normal Hb was not affected by buffer conditions. But des-Arg α 141-des-Hisβ146 Hb in the presence of IHP showed a blue-shift by about 1.5 nm relative to the spectrum in the absence of IHP ( compare f1 with f2 and f3 spectra ).

Figure 1C shows the difference CD spectra between the CD spectra of reconstituted normal Hb and the arithmetic means of the spectra of normal α and β chains ( spectra g = e − (a+b)/2 ), and between the CD spectra of reconstituted des-Argα141-des-Hisβ146 Hb and the arithmetic means of the spectra of des-Argα141 α chain and des-Hisβ146 β chain ( spectra h = f − (c+d)/2 ). The intensity of the difference CD band for normal Hb was somewhat affected by buffer conditions, while their peak position was not. A blue-shift in the peak position induced by IHP was found in des-Argα141-des-Hisβ146 Hb ( compare h1 with h2 and h3 spectra ) : the wavelengths of the CD peak in the presence and absence of IHP were 433±1 nm and 437±1 nm, respectively.

It is well-known that the Soret CD spectrum of reconstituted normal Hb is remarkably different from the arithmetic mean of respective spectra of its constituent chains [11]. This difference has been shown to be due to the interaction between α1 and β1 subunits to make the α1β1 dimer [12-15]. However, it is not clear that the difference between the CD spectra of reconstituted modified Hb and the arithmetic means of those of their constitutent chains is ascribed to the same interaction. In order to elucidate this problem, the Soret CD change after mixing of modified α and β chains was investigated kinetically with a CD stopped-flow apparatus.

CD Stopped-Flow Measurements Figure 2 shows a typical trace of the CD change in the Soret region after mixing of equimolar concentrations of des-Argα141 α chain and des-Hisβ146 β chain in the absence of IHP at pH 6.0. The time course gave a straight line in the second-order plots as shown in Figure 2 inset. The value of the slope of the straight line in the second-order plots increased linearly with an increase in the protein concentration, giving one straight line ( Fig.3 ). The value of the second-order rate constant was obtained as 2.75 x $10^5$ $M^{-1}s^{-1}$ regardless of kinds of modified chains or addition of IHP ( Fig.3 ). This value is comparable with a value of 6.4 x $10^5$ $M^{-1}s^{-1}$ for normal Hb at pH 7.5 and 25°C [12]. These results indicate that the Soret CD change after mixing of modified α and β chains obeyed the second-order reaction rate law, that is, the difference between the Soret CD spectra of the reconstituted modified Hb's and the arithmetic means of respective spectra of their constituent chains was induced by the interaction between α1 and β1 subunits to make the α1β1 dimer. Therefore, the difference CD spectra for modified Hb's which have been identified previously as the R or T structures will provide significant informations about the relationship between the quaternary structure and the α1β1 interaction. Then, we investigated the difference CD spectra

② 

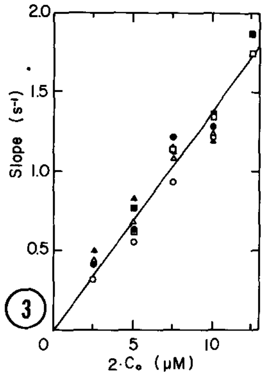


Figure 2. Time course of the CD change at 437 nm after mixing of equimolar concentrations of des-Argα141 α chain and des-Hisβ146 β chain in 0.1 M Bis-Tris ( pH 6.0 ), 0.1M $Cl^-$ at 15°C. The CD traces are of the average of 16 runs. The initial concentrations of both the chains ( after mixing ), $C_0$, were 10 μM. Initial level is the arithmetic mean of the CD of des-Argα141 α chain and des-Hisβ146 β chain, obtained with a computer. The inset shows second-order plots of the time course in Fig. 2. The ordinate H is given by $\Delta CD_f/(\Delta CD_f - \Delta CD)$ where $\Delta CD_f$ is total CD increases, ΔCD is the CD increase at time t.

Figure 3. Relationship between the slope of the second-order plots of the CD change and the concentation of the chains ( after mixing ), $C_0$. ( ○ , ● ); normal Hb, ( △ , ▲ );des-Argα141-des-Hisβ146 Hb, ( □ , ■ ); des-Argα141-Tyrα140-des-Hisβ146-Tyrβ145 Hb.
The Buffer conditions are 0.1 M Bis-Tris (pH 6.0), 0.1 M $Cl^-$ in the absence of IHP (open symbols), and in the presence of 1 mM IHP (closed symbols).

of eight kinds of modified Hb's in the presence and absence of IHP at pH 6.0 and at pH 9.0, as follows.

The Difference CD Spectra The peak wavelength of the difference CD spectra could be classsified into two groups, one was 433±1 nm and the other 437±1 nm ( Table I ). The peak intensity of the difference CD spectra decreased with an increase in the number of removed residues ( data not shown ). The quaternary structures previously identified [4-10] are also given in Table I. A comparison of the classification with the quaternary structures revealed that the R and T structures showed a maximum of the difference CD spectra at about 437 nm and 433 nm, respectively, except des-Hisβ146-Tyrβ145 Hb at pH 6.0. Since the difference CD between the CD spectra of the reconstituted modified Hb's and the arithmetic means of those of their constitutent chains can be ascribed to the α1β1 interaction as mentioned above, the difference in the peak wavelength of the difference CD spectra must reflect the difference in the α1β1 interaction. Hsu and Woody showed [17] that the

Table I. The wavelength of maximum of the difference CD spectra between the CD spectra of reconstituted modifed Hb's and the arithmetic means of respective spectra of their constituent chains. Each wavelength includes an error of ± 1 nm. R and T indicate the quaternary structure of the modified Hb characterized previously. (R) and (T) are the quaternary structure predicted from the present work.

| | | pH 6.0 | | pH 9.0 | |
|---|---|---|---|---|---|
| $\alpha$ chain | $\beta$ chain | + IHP | - IHP | - IHP | ref. |
| normal | normal | 433 T | 433 T | 433 T | 7 |
| | des-His | 433 T | 433 T | 433 T | 5,6,7,10 |
| | des-His-Tyr | 433 T | 433 R | 433 (T) | 5,8,9 |
| des-Arg | normal | 433 T | 433 T | 437 R | 6,7,9,10 |
| | des-His | 433 T | 437 R | 437 (R) | 6,7,10 |
| | des-His-Tyr | 433 (T) | 437 (R) | 437 (R) | |
| des-Arg-Tyr | normal | 433 T | 437 R | 437 R | 4,7,9 |
| | des-His | 433 (T) | 437 (R) | 437 (R) | |
| | des-His-Tyr | 437 (R) | 437 (R) | 437 (R) | |

Soret CD spectra of Hb originate from the interaction between $\pi$-$\pi^*$ transitions of heme and aromatic residues located at a distance of about 12 Å from the heme. Therefore, the difference in the peak wavelength may be ascribed to the difference in the tertiary structural changes in the regions including these residues upon assembling of $\alpha$ and $\beta$ chains to make the $\alpha 1\beta 1$ dimer. On the basis of the peak wavelength of the difference CD spectrum in the modified Hb's whose quaternary structures are unknown, we predicted their quaternary structures and added them in Table I.

It has been emphasized that the interaction between the $\alpha 1$ and $\beta 2$ subunits is more important than that between the $\alpha 1$ and $\beta 1$ subunits for the quaternary structure of Hb molecule, because on oxygenation larger movements occur at the former [18,19]. Our results suggest that the tertiary structure in the heme vicinity, which are characteristic of R and T structures, is mostly achieved upon the formation of $\alpha 1\beta 1$ dimer before the construction of $\alpha_2\beta_2$ tetramer. This implies that the $\alpha 1\beta 1$ interaction is also of great importance to understand the structure-function relationship of Hb molecule.

## ACKNOWLEDGEMENTS

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