NUCLEAR MAGNETIC RESONANCE STUDIES OF HEMOGLOBIN. IV. THE STRUCTURE-FUNCTION RELATIONSHIP OF HUMAN ADULT HEMOGLOBINS A AND CHESAPEAKE AND ITS IMPLICATION TO THE NATURE OF OXYGENATION OF HEMOGLOBIN*

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Summary

Nuclear magnetic resonance spectra of Hb A and Hb Chesapeake in both carboxy- and deoxy-forms suggest that (1) there are differences in the aromatic proton resonances between these two proteins which may be due to the altered interactions among the amino acid residues at α_1-β_2 contacts as a result of the amino acid substitution at α-92 in Hb Chesapeake, and (2) amino acid residues at FG3 (leucine) and/or FG5 (valine) may be responsible for transmitting information from α_1 to β_2 chain or vice versa in the course of oxygenation of hemoglobin.

Many of the functional properties of hemoglobin are insensitive to replacement of most amino acid residues on its surface but they may be profoundly altered by relatively small changes of internal non-polar contacts, such as those near the heme and those contacts between the subunits[1]. For this reason, hemoglobin Chesapeake (Hb Chesapeake) is a suitable genetic variant to gain some insight into the structure-function relationship of hemoglobin. The position of amino acid substitution in Hb Chesapeake is α-92 (FG4), i.e., arginine is replaced by leucine[2], which is located at α_1-β_2 contacts[1,3]. According to Nagel, Gibson, and Charache[4], this abnormal hemoglobin is characterized by a high oxygen affinity, a decreased or absent subunit interaction, and a normal Bohr effect. All known human hemoglobin variants which have amino acid substitutions at α_1-β_2 contacts exhibit diminished subunit interactions[1].

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Our basic approach to the understanding of the mechanism of oxygenation of hemoglobin is to use physical techniques, such as nuclear magnetic resonance (NMR), to pinpoint the structural differences between normal adult and abnormal hemoglobins and to attempt to relate these differences in structure to the altered functional properties of these mutant hemoglobins.

Experimental

Materials: In some experiments, Hb A was prepared from fresh human blood obtained from the local Blood Bank by the usual procedure and was lyzed either by toluene or distilled water. In other studies, hemoglobins A and Chesapeake were isolated from heterozygotes. Red cells were lyzed with distilled water and CCl_4, and hemoglobins A and Chesapeake were separated by chromatography on DEAE-Sephadex[5]. Deoxyhemoglobin was prepared from HbCO by first passing oxygen to remove CO and then passing oxygen-free nitrogen or argon to remove oxygen. As judged by visible spectrum, deoxyhemoglobin samples used in our study were better than 95% in the deoxy-form and contain less than 2% methemoglobin. Hemoglobin samples for NMR studies were prepared in 0.1 M deuterated phosphate at pD $\sim$ 7 as described earlier[6]. pD readings were obtained directly from a Radiometer pH meter Model 26 in conjunction with a Beckman Model 39036 frit junction combination electrode plus 0.4 unit.

Methods: A Bruker HFX-3 NMR spectrometer operating at a frequency of 90 MHz was used to obtain NMR spectra. The ambient temperature of the probe was 28°C. We have used the residual water in each sample as the internal reference for calibrating the proton chemical shifts. The value for HDO is -4.72 ± 0.01 ppm (i.e., down-field) from the methyl resonance of sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) from pD 6.55 to 7.68. The signal to noise ratios in some cases were improved by using a Fabri-Tek Model 1062 or 1074 time averaging computer.

Results

The proton NMR spectra of HbCO A and HbCO Chesapeake are very similar

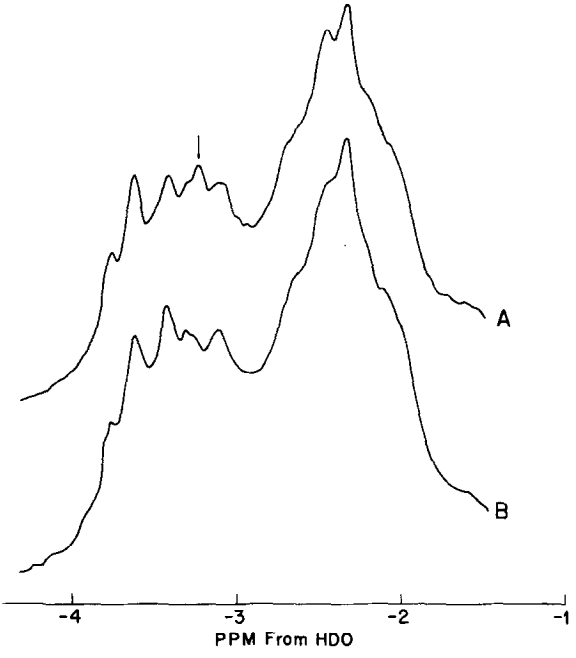

Figure 1. 90 MHz proton NMR spectra of carboxyhemoglobins A and Chesapeake over the aromatic proton resonance region: A, 5% Hb A at pD 6.9; and B, 7% Hb Chesapeake at pD 7.0.

except over the aromatic proton resonance region. There are observable differences between HbCO A and HbCO Chesapeake from -1 to -4 ppm from HDO. In particular, the resonance at -3.20 ppm is of less intensity or missing in HbCO Chesapeake. Figure 2 gives the 90 MHz proton NMR spectra of Hb A and Hb Chesapeake in the deoxy form over the aromatic proton resonance region. These spectra show differences between these two proteins. In particular, the resonances at about -3.63 and -3.05 ppm are apparently of less intensity or missing in Hb Chesapeake. Since the aromatic amino acid residues are identical between Hb A and Hb Chesapeake, the differences in the aromatic proton resonances between these two hemoglobins reflect a difference in the conformation between these two proteins. In addition, the differences in the aromatic proton resonances (Figures 1 and 2) between carboxy- and deoxy-forms

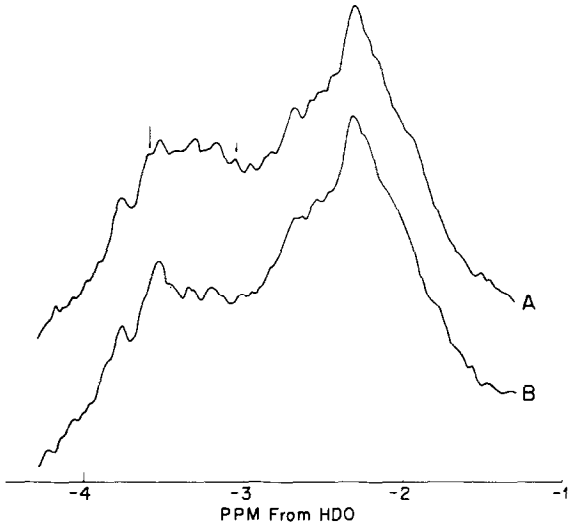

Figure 2. 90 MHz proton NMR spectra of deoxyhemoglobins A and Chesapeake over the aromatic proton resonance region: A, 5% Hb A at pD 6.9; and B, 5% Hb Chesapeake at pD 6.9.

of the respective hemoglobins indicate that there is a conformational change in going from deoxy- to carboxyhemoglobin.

Figure 3 gives the 90 MHz proton NMR spectra of some of the aliphatic proton resonance region (i.e., from +2 to +7 ppm from HDO) of Hb A and of Hb Chesapeake in both carboxy- and deoxy-forms. The proton resonances of Hb A over this region are very similar to those of Hb Chesapeake. For a diamagnetic protein such as HbCO, the only proton resonances expected above +4.0 ppm from HDO are those due to the ring-current shifted resonances[7-9]. These so-called ring-current shifted resonances are due to some of the protons of the amino acid residues which are in close juxtaposition to the faces of the porphyrin rings or the aromatic amino acid residues in hemoglobin so that they experience ring-current magnetic fields which will shift resonances to higher field[7]. According to Phillips and coworkers[7,10], ring-current shifted resonances in excess of +6.7 ppm from HDO are most likely due to the porphyrin rings rather than those of aromatic rings in a protein.

There are four such weak resonances at +4.83, +5.47, +5.89, and +6.60 ppm from HDO in both HbCO A and HbCO Chesapeake. It is reasonable to assume that most of the ring-current shifted resonances shown in Figure 3 arise from the porphyrin rings of hemoglobin. The resonance at +4.83 ppm appears only to decrease in relative intensity in going from deoxy- to carboxyhemoglobin. Upon deoxygenation, the resonances at +5.47, +5.89, and +6.60 ppm disappear in both Hb A and Hb Chesapeake.

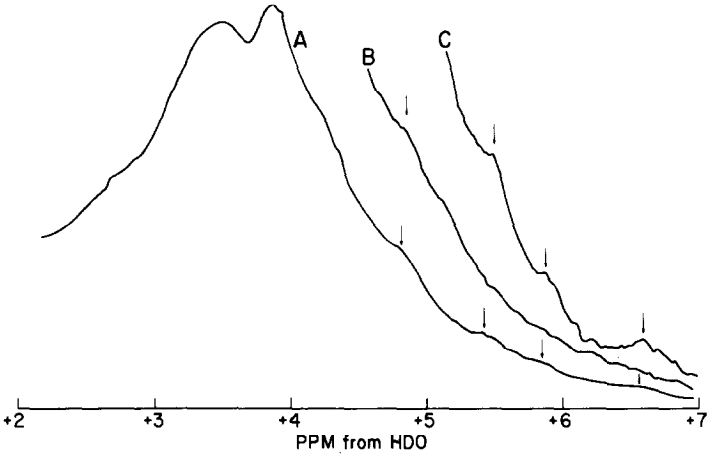

Figure 3. 90 MHz proton NMR spectra of Hb A and Hb Chesapeake in carboxy- and deoxy-forms over part of the aliphatic proton resonance region: A, 11% HbCO A at pD 7.1; B, expanded scale of 5% deoxy-Hb Chesapeake at pD 6.9; and C, expanded scale of 7% HbCO Chesapeake at pD 7.0.

Discussion

According to the x-ray crystallographic results of Perutz, et al[3,11], the contacts among the amino acid residues between α_1-β_2 subunits are of primary importance because they contain several side chains forming bridges between residues which touch the heme on either side (such as FG3α_1, FG5α_1, and FG5β_2), thus offering a direct pathway for interactions between two pairs of hemes which are closest together (25 Å between the two iron atoms). Fur-

thermore, the greatest relative movement takes place between α_1-β_2 subunits upon oxygenation[3,12]. On the other hand, the interactions between the heme groups of α_1 and β_1 subunits have to be transmitted through an intricate network of many residues acting over a much longer distance (37 Å between the two iron atoms)[3,11].

Perutz's model and stero-drawings of hemoglobin[3] show that the methyl or methylene groups of the following residues, E11(valine), F4(leucine), F7 (leucine), FG3(leucine), and FG5(valine), are likely to be the ones which give rise to the ring-current shifted resonances shown in Figure 3. All these residues are in close proximity to the heme groups in both α and β chains[3]. Amino acid residues at FG3 and FG5 are not only very close to the heme group but are also located at α_1-β_2 contacts[3]. Hence, any conformational change in these two residues not only may affect the conformation of those residues located at α_1-β_2 contacts but also may propagate this conformational change to the other heme group. The change in the appearance of the ring-current shifted resonances in going from deoxy- to carboxy forms in both Hb A and Hb Chesapeake (Figure 3) suggests that there is a conformational change in these critical amino acid residues.

In Hb A, arginine (FG4α_1) is in van der Waals contact with tryptophan (C3β_2), glutamine (C5β_2), and arginine (C6β_2)[1,3]. The guanidinium group of arginine in FG4α_1 may also make a hydrogen bond with a recipient group in the β_2 chain[1,3]. The substitution of this arginine by a non-polar amino acid leucine will alter the interactions between this residue and those at C3, C5, and C6 of the β_2 subunit. These residues in turn are in van der Waals contact with other amino acid residues so that the replacement at FG4α_1 could conceivably alter numerous interactions in the α_1-β_2 contact region. Tryptophan (C3β_2) contacts valine (FG5α_1), aspartic acid (G1α_1), proline (G2α_1), and tyrosine (H23α_1)[1,3]. Arginine (C6β_2) contacts threonine (C6α_1), tyrosine (C7α_1), and leucine (FG3α_1)[1,3]. Threonine (C6α_1) makes a hydrogen bond with histidine at FG4β_2[1,3]. This histidine makes van der Waals contacts with

threonine at C3α_1 which in turn contacts valine at FG5β_2[1,3]. The two contacts with tyrosines (C7α_1 and H23α_1) are especially interesting because spectrophotometric titrations of the tyrosines in deoxy Hb A and deoxy Hb Chesapeake suggest that two tyrosine residues in deoxy Hb A are different from those in deoxy Hb Chesapeake[4].

It is attractive to speculate that the altered aromatic proton resonances (Figures 1 and 2) in Hb Chesapeake may be due to the altered interactions between leucine at FG4α_1 with aromatic amino acids, such as tryptophan (C3β_2), tyrosines (C7α_1 and H23α_1), and histidine (FG4β_2). These altered interactions as well as the elimination of a hydrogen bond could interfere with structural transitions in going from deoxy- to oxyhemoglobin Chesapeake. In other words, proper interactions among those amino acid residues located at α_1-β_2 contacts may be important for the physiologic function of hemoglobin as an oxygen carrier.

In conclusion, we wish to suggest that leucine at FG3 and/or valine at FG5 may be involved in transmitting information from the α_1 chain to the β_2 chain or vice versa during oxygenation of hemoglobin. Further work is needed to elucidate how information is transmitted to the four subunits in hemoglobin and to determine if a tetramer or a dimer is the functional unit in hemoglobin. We have also suggested a mechanism whereby the substitution of arginine at FG4α_1 by a leucine in Hb Chesapeake could alter the normal interactions among the amino acid residues located at α_1-β_2 contacts essential for the physiologic function of hemoglobin. Our present NMR results are consistent with Perutz's model of hemoglobin.

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