Nuclear Magnetic Resonance Studies of Hemoglobin Chesapeake: An $\alpha_1\beta_2$ Mutant†

Karen J. Wiechelman, Samuel Charache, and Chien Ho*

ABSTRACT: Proton nuclear magnetic resonance studies have been used to compare the deoxy and ligand-bound forms of hemoglobins A and Chesapeake (α92FG4 Arg →Leu). Partial saturation studies have also allowed us to study the equilibrium binding of oxygen and carbon monoxide to Hb Chesapeake. The hyperfine shifted resonances of the heme protons of hemoglobins Chesapeake and A differ, indicating that the deoxy quaternary structure of hemoglobin Chesapeake has been altered in some way. The ring-current shifted proton resonances of both HbCO and HbO_2 Chesapeake are identical with those of the corresponding ligand-bound forms of Hb A suggesting that the tertiary structures of the α and β heme pockets in liganded Hb Chesapeake are very similar to those of liganded Hb A. Partial CO and O_2 binding studies show that in Hb Chesapeake the hemoglobin-ligand reaction is both ligand and phosphate specific. These studies also show that the α and β chains within the Hb Chesapeake tetramer are functionally more equivalent in their reactions with ligands than are the α and β chains of Hb A.

It has been shown that hemoglobins with mutations at the $\alpha_1\beta_2$ subunit interface exhibit decreased heme-heme interactions (Perutz and Lehmann, 1968) indicating that the structural integrity of this region of the hemoglobin (Hb)[1] molecule is critical for cooperative interactions in normal Hb A. X-Ray crystallographic studies have shown that the transition from the deoxy to the oxy quaternary structure results in a drastic change in the $\alpha_1\beta_2$ interface. On oxygenation the two subunits shift by 13° relative to each other resulting in the breaking of the hydrogen bond between tyrosine-α42(C7) and aspartic acid-β99(G1) which helps stabilize the deoxy structure, and the formation of a new hydrogen bond between aspartate-α94(G1) and asparagine-β102(G4) in the oxy quaternary structure (Perutz, 1970; Perutz and Ten Eyck, 1971). The deoxy to oxy quaternary structural change results in a slight loosening of the $\alpha_1\beta_1$ subunit interface but the two subunits do not shift relative to each other (Perutz and Ten Eyck, 1971). The $\alpha_1\beta_2$ interface is both smaller and smoother than the $\alpha_1\beta_1$ subunit interface and it is constructed so that the subunits can easily slide past one another. It is closely connected to the heme groups so that any changes in the $\alpha_1\beta_2$ subunit interface would be expected to affect the heme environment (Perutz, 1969). Thus studies of hemoglobins with mutations at the $\alpha_1\beta_2$ interface have proved to be invaluable in the study of structure-function relationships in hemoglobin.

Hb Chesapeake is an α chain mutant with a leucine residue replacing the normal arginine residue at α92(FG4). This substitution results in a higher than normal oxygen affinity, a normal Bohr effect, and a reduced cooperativity which is reflected in the Hill coefficient (n) of 1.3 (Nagel *et al.*, 1967) as compared to a value of 2.9 for Hb A (Antonini *et al.*, 1964). In the presence of organic phosphates, such as 2,3-diphosphoglycerate (DPG) or inositol hexaphosphate (IHP), the oxygen affinity of Hb Chesapeake is reduced and the cooperativity is increased (Imai, 1974).

Proton nuclear magnetic resonance (nmr) studies have shown that there are three prominent hyperfine (or contact) shifted resonances in deoxy Hb A (Davis *et al.*, 1971; Ogawa and Shulman, 1972). Unpaired electrons of the paramagnetic iron interact with protons of the porphyrin ring and/or with the amino acid protons that are positioned sufficiently close to the iron atom to give resonances at −17.6, −12.0, and −7.9 ppm from the residual HDO resonance (Davis *et al.*, 1971). Nmr studies of various mutant hemoglobins have shown that the resonance at −17.6 ppm can be assigned to a β chain proton while the resonances at −12.0 and −7.9 ppm are due to protons on the α chain (Davis *et al.*, 1971; Lindstrom *et al.*, 1972a). These hyperfine shifted resonances return to their normal positions buried in the aromatic or aliphatic regions of the Hb spectrum when ligands bind to the hemes making them diamagnetic.

The diamagnetic forms of hemoglobin (HbCO and HbO_2) contain ring-current shifted proton resonances that are due to protons of amino acids that are positioned closely enough to the porphyrin ring to be affected by the local magnetic fields which are produced by the delocalized π electrons in the heme groups (McDonald and Phillips, 1967; McDonald *et al.*, 1969; Shulman *et al.*, 1970). These ring-current shifted resonances have been shown to be very sensitive to changes in the tertiary structure around the ligand binding sites in HbCO (Lindstrom *et al.*, 1972b; Lindstrom and Ho, 1973).

In this communication we report the results of nmr studies of the hyperfine and ring-current shifted proton resonances of Hb Chesapeake.

Experimental Section

Materials. Hb Chesapeake was isolated and purified by chromatography on DEAE-Sephadex (A 50 Pharmacia)

† From the Department of Biophysics and Microbiology and the Department of Biochemistry, Faculty of Arts and Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15260 (K.J.W. and C.H.), and the Hematology Division, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205 (S.C.). *Received June 4, 1974.* Supported by research grants from the National Institutes of Health (HL-10383; HL-02799; RR-00292), National Science Foundation (GB-37096X), and the American Heart Association, Inc.

[1] Abbreviations used are: Hb, hemoglobin; DPG, 2,3-diphosphoglycerate; IHP, inositol hexaphosphate; HbCO, carbonmonoxyhemoglobin; HbO_2, oxyhemoglobin; Tris, tris(hydroxymethyl)aminomethane; Bis-Tris, 2,2-bis(hydroxyethyl)-2,2′,2″-nitrilotrimethanol.

using the isolation and purification procedure of Huisman and Dozy (1965). Hb A was isolated either from the Hb Chesapeake hemolysate or was prepared by standard methods from fresh whole blood samples obtained from the local blood bank (Davis *et al.*, 1971). Hemoglobin was freed from phosphates by passing it through a column of Sephadex G-25 (Pharmacia) equilibrated with 0.01 M Tris-HCl buffer containing 0.1 M NaCl at pH 7.5 (Berman *et al.*, 1971). To reduce the intense proton resonance of aqueous samples, H_2O was exchanged with deuterium oxide (Merck, Sharp, and Dohme of Canada, Ltd.) by repeated dilution with D_2O and subsequent concentration through an Amicon UM-20E membrane. DPG was purchased from Calbiochem as the pentacyclohexylammonium salt, converted to the acid form with Bio-Rad AG50W-X8 cation exchange resin, and titrated to pH 7 with NaOH. IHP purchased from Sigma as the sodium salt was dissolved in D_2O and titrated to pD 7 with HCl. Both the DPG and IHP solutions were then repeatedly lyophilized and redissolved in D_2O. After the final lyophilization the residue was dissolved in D_2O and the phosphate concentration of the solutions was determined (Bartlett, 1950). 2,2-Bis(hydroxyethyl)-2,2′,2″-nitrilotrimethanol (Bis-Tris) purchased from Aldrich was dissolved in D_2O and titrated to pD 7 with HCl. The pD of all solutions was determined by adding 0.4 pH unit (Glasoe and Long, 1960) to the meter reading of a Radiometer Model 4 pH meter equipped with a Beckman 39030 combination electrode. Oxyhemoglobin was prepared from HbCO by replacing the CO with oxygen by flushing oxygen over the Hb solution contained in a round-bottomed flask attached to a rotatory evaporator in an ice-water bath under a Sylvania 150-W flood lamp. Oxygen was removed by flushing the solution with prepurified nitrogen. The hemoglobin concentrations for our nmr studies varied from 9 to 14%.

For the oxygen or carbon monoxide saturation studies, partially liganded samples were prepared by one of two methods. When the study was carried out in 5-mm nmr sample tubes, appropriate amounts of deoxy Hb were mixed with either HbCO or HbO_2 to give partially liganded samples. However, in the case of the oxygen saturation study in the presence of IHP, the experiments were carried out in the presence of the methemoglobin reductase system of Hayashi *et al.* (1973) with NADPH substituted for NADP. All of the materials used in the reductase system were purchased from Sigma. Appropriate amounts of air were injected into 5-mm tubes containing deoxy Hb Chesapeake and the sample was equilibrated for at least 30 min before nmr spectra were taken. In the 10-mm sample tubes partially liganded samples were obtained by injecting small amounts of oxygen or carbon monoxide gas with a Hamilton microsyringe through a rubber stopper in the side arm of the tube. The samples were allowed to equilibrate for at least 1.5 hr before nmr spectra were taken. The degree of ligand saturation was measured by monitoring the decrease in the deoxy peak at 757 nm with either a Cary 14 or Zeiss PMQ II spectrophotometer. For the 5-mm sample tubes the absorbance was determined directly through the nmr tubes held in the light path of a specially designed holder. A piece of flat glass tubing (2–3 mm in optical path length) was fused to the top of the 10-mm nmr tubes to serve as a cuvet.

Methods. Nmr spectra were obtained with 5-mm sample tubes on MPC-HF 250-MHz superconducting spectrometer (Dadok *et al.*, 1970) and on a Bruker HFX 90-MHz spectrometer with the 10-mm sample tubes. Proton chemical

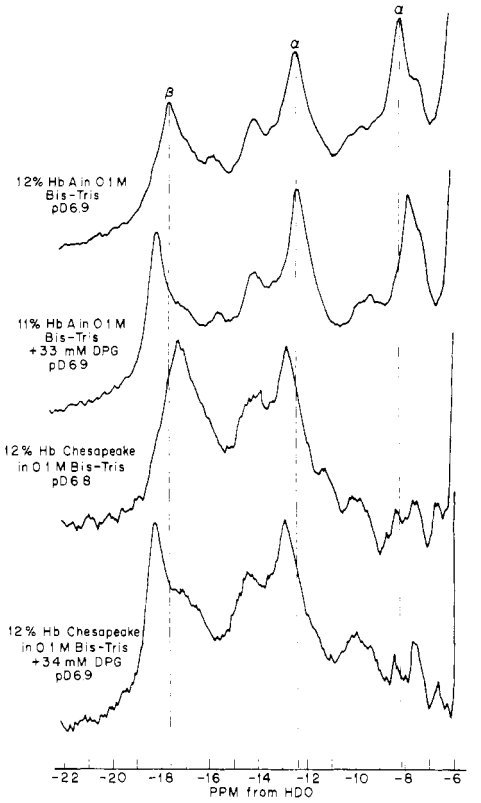

FIGURE 1: The 250-MHz proton nmr spectra of deoxyhemoglobin A in 0.1 M Bis-Tris and 0.1 M Bis-Tris plus 33 mM DPG at pD 6.9, and deoxyhemoglobin Chesapeake in 0.1 M Bis-Tris (pD 6.8) and 0.1 M Bis-Tris plus 34 mM DPG (pD 6.9) at 31°. The symbols α and β above the peaks indicate those resonances assigned to α and β hemes.

shifts are referenced with respect to the residual H_2O in the sample. Ambient temperatures of the spectrometers were 31° for the 250 MHz and 28° for the 90 MHz. The HDO signal is −4.72 ppm downfield from the proton resonance of 2,2-dimethyl-2-silapentane-5-sulfonate at 31° and −4.80 ppm at 28°. The negative sign of the chemical shifts indicates that the resonance is downfield from HDO and a positive sign indicates that the resonance is upfield from the HDO signal. The chemical shifts of the hyperfine shifted resonances are accurate to ±0.2 ppm and the chemical shifts of the ring-current shifted resonances are accurate to ±0.05 ppm. Signal to noise ratios were improved by signal averaging with a Fabri Tek 1074 computer when using the Bruker spectrometer and by the nmr correlation spectroscopy technique with a Sigma-5 computer which was interfaced to the MPC-HF 250-MHz spectrometer (Dadok *et al.*, 1972).

Results

In Figure 1 the hyperfine shifted proton resonances of deoxy Hb A and deoxy Hb Chesapeake are compared. The hyperfine shifted spectrum of Hb A shows three prominent low-field proton resonances while the deoxy Hb Chesapeake spectrum has only two major hyperfine shifted resonances with the α chain resonance at −7.9 ppm missing as was first shown by Davis *et al.* (1971). The other two resonances are found at −17.4 and −12.8 ppm in deoxy Hb Chesapeake and at −17.6 and −12.0 ppm in the deoxy Hb A spectrum. The addition of organic phosphates to deoxy Hb A in 0.1 M Bis-Tris buffer at pD 7 causes shifts in the positions of the hyperfine shifted resonances: β-heme resonance is shifted ~0.5 ppm downfield and the α resonances are shifted ~0.1

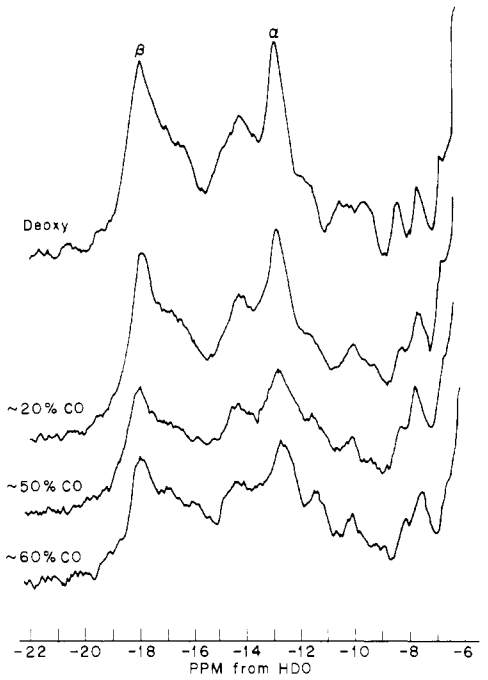

FIGURE 2: The 250-MHz proton nmr spectra of 10% Hb Chesapeake in 0.13 M potassium phosphate at pD 6.9 and 31° as a function of CO saturation.

ppm unfield (Ho *et al.*, 1973). The addition of DPG or IHP to deoxy Hb Chesapeake in 0.1 M Bis-Tris at pD 7 results in corresponding shifts in the β-heme resonance and in the α-heme resonance at -12.8.

We have not been able to reproduce the results of an earlier study which indicated that CO binds preferentially to the α hemes of Hb Chesapeake in 0.1 M potassium phosphate buffer at pD 7 (Davis *et al.*, 1971). This result might have been due to methemoglobin formation in the sample since methemoglobin Chesapeake formation in the presence

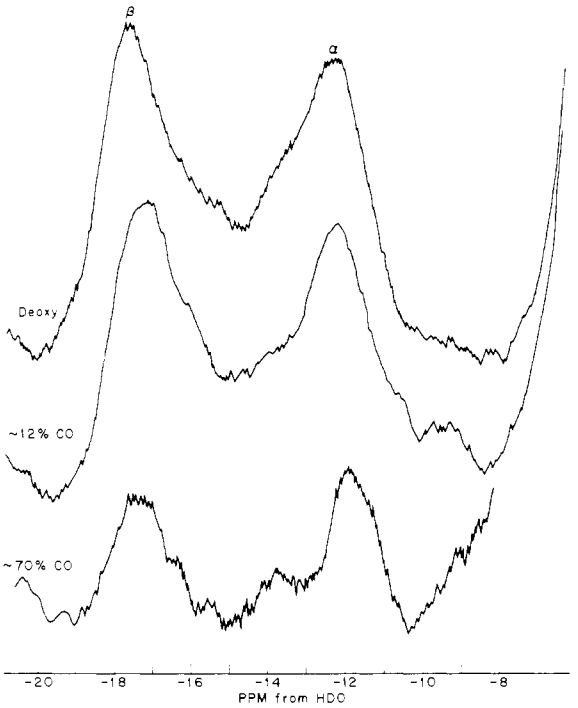

FIGURE 3: The 90-MHz proton nmr spectra of 9% Hb Chesapeake in 0.1 M Bis-Tris plus 32 mM DPG as a function of CO saturation at pD 6.9 and 28°.

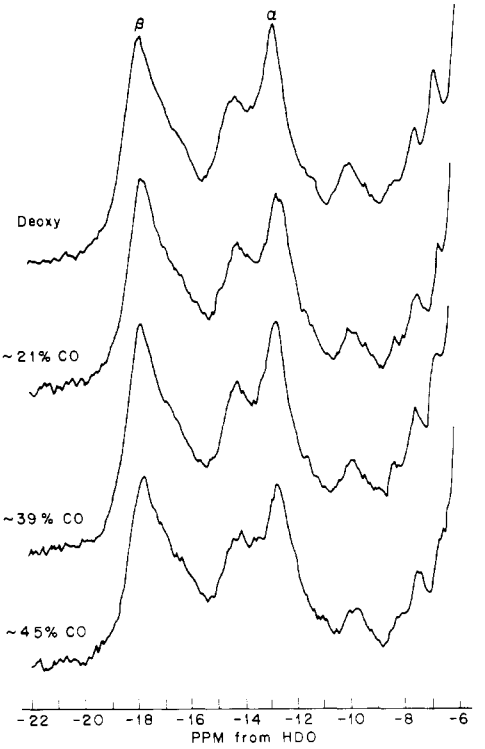

FIGURE 4: The 250-MHz proton nmr spectra of 12% Hb Chesapeake in 0.14 M Bis-Tris plus 13 mM IHP at pD 6.8 and 31° upon increasing CO saturation.

of organic phosphates results in a preferential decrease in the area under the α-heme resonance (K. J. Wiechelman and C. Ho, unpublished results). The results of the present study show that CO binds randomly to the α and the β hemes in Hb Chesapeake in 0.13 M potassium phosphate, 0.1 M Bis-Tris, 0.1 M Bis-Tris + 32 mM DPG, and 0.14 M

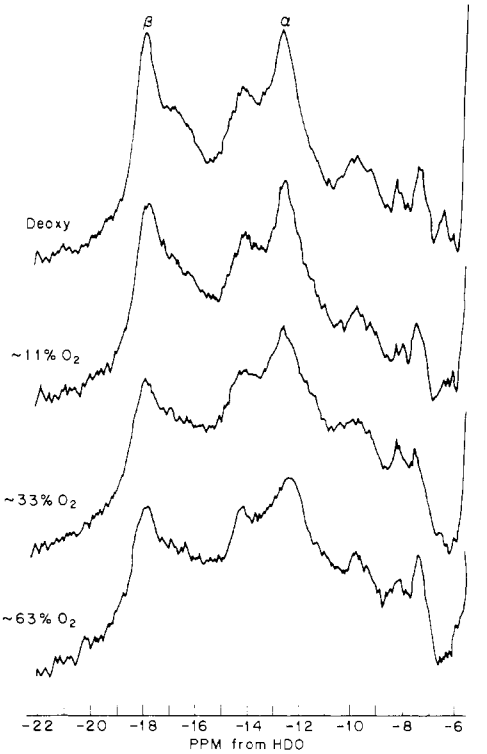

FIGURE 5: The 250-MHz proton nmr spectra of 10% Hb Chesapeake in 0.1 M Bis-Tris plus 34 mM DPG at pD 6.9 and 31° as a function of O_2 saturation.

FIGURE 6: The 250-MHz proton nmr spectra of 10% Hb Chesapeake in 0.1 M Bis-Tris plus 9 mM IHP in the presence of methemoglobin reductase upon increasing O_2 saturation at pD 6.7 and 31°.

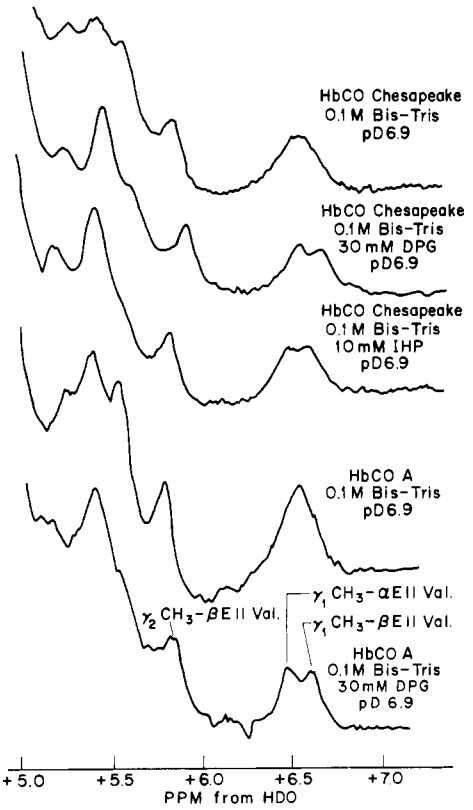

FIGURE 7: The 250-MHz nmr spectra of the ring-current shifted proton resonances of HbCO Chesapeake and HbCO A in the absence and presence of organic phosphates at 31°. The assignments of the valine E11 methyl groups of HbCO A are from Lindstrom *et al.* (1972b).

Bis-Tris + 13 mM IHP, all at pD 7 (Figures 2-4). If there is preferential ligand binding to either the α or β hemes in Hb Chesapeake, one would observe a selective decrease in the intensity of either the α or the β chain resonances. On the contrary the area ratio of the α resonance (at ~ −12.8 ppm) and the β resonance (at ~−17.6 ppm) remains essentially constant as a function of CO saturation (Figures 2-4). This suggests that CO binds essentially randomly to the α and β chains of Hb Chesapeake. A detailed procedure for determining preferential binding of ligands to Hb A by the nmr technique has been described by Ho and coworkers (Lindstrom and Ho, 1972; Johnson and Ho, 1974).

Figures 5 and 6 show the results of oxygen saturation studies for Hb Chesapeake. When HB Chesapeake is in 0.1 M Bis-Tris buffer or in 0.1 M Bis-Tris + 34 mM DPG at pD 7, oxygen binds to the α and β hemes essentially randomly as evidenced by the lack of a selective decrease in the area of either the α- or the β-heme resonance upon oxygenation (Figure 5). In the presence of 9 mM IHP oxygen binds to the α hemes of Hb Chesapeake in preference to the β hemes as shown by the preferential decrease of the α-heme resonance at ~−12.8 ppm as a function of oxygenation (Figure 6). In other words, the area ratio of the α to β resonances decreases upon oxygenation. Deoxy Hb Chesapeake is unstable in the presence of IHP and in the presence of oxygen significant amounts of methemoglobin were formed during the course of the experiment. The methemoglobin formation could be prevented by the addition of the methemoglobin reductase system of Hayashi *et al.* (1973). The reductase system in D_2O will reduce a 10% solution of methemoglobin in about 45 min (when NADPH is introduced rather than being generated from NADP by glucose-6-phosphate dehydrogenase) so it can efficiently reduce the methemoglobin formed in the sample during the course of an nmr experiment. This reductase system was used by Imai (1974) to carry out oxygen equilibrium measurements on Hb Chesapeake. The reductase system was also included in oxygen saturation experiments with Hb A in the presence of 10 mM IHP and Hb Chesapeake in the presence of 30 mM DPG. In both cases the results were the same as those obtained with samples which contained no reductase.

The ring-current shifted proton resonances of HbCO Chesapeake and HbO_2 Chesapeake in 0.1 M Bis-Tris, in the presence of 30 mM DPG, and in the presence of 10 mM IHP are very similar to the corresponding ring-current shifted resonances of HbCO A and HbO_2 A (Figures 7 and 8). This suggests that the tertiary structures of the α- and β-heme pockets in liganded Hb Chesapeake are very similar to those of liganded Hb A.

Discussion

X-Ray crystallographic studies of deoxy Hb Chesapeake at 5.5-Å resolution indicate that Hb Chesapeake can assume a deoxy quaternary structure which is similar to that of deoxy Hb A (Greer, 1971). The difference electron density map of deoxy Hb Chesapeake and deoxy Hb A shows no significant changes other than the missing electron density of the arginine residue at the mutation site. When air is introduced into the deoxy crystals, the crystals become highly disordered which indicates that a quaternary structural change accompanies the deoxy to oxy transition in Hb Chesapeake (Greer, 1971). Crystals of oxy Hb Chesapeake are isomorphous with oxy Hb A crystals but the difference Fourier between the two hemoglobins shows that the overall conformations are not the same. The B, C, D, and G helicies of the same β chain which are in close proximity to the α92(FG4) mutation site appear to have moved away from

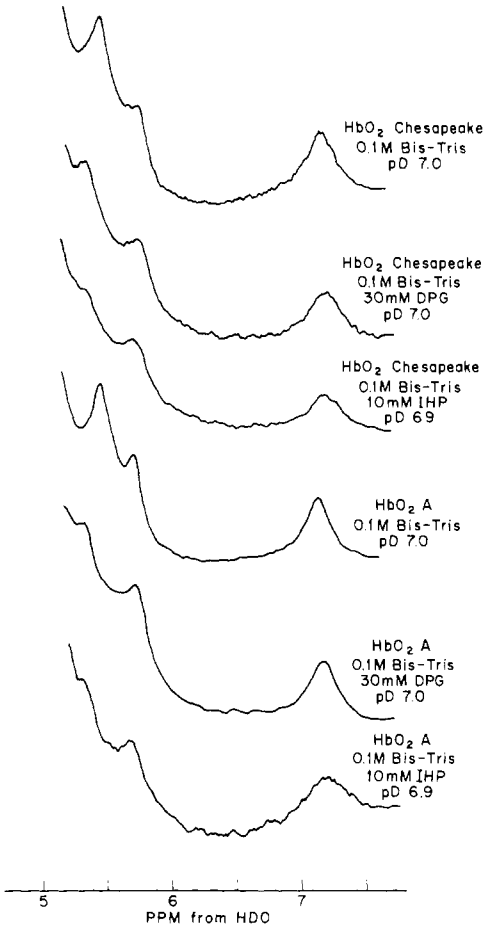

FIGURE 8: The 250-MHz nmr spectra of the ring-current shifted proton resonances of HbO_2 Chesapeake and HbO_2 A and the changes brought about in these resonances by the addition of DPG or IHP.

the leucine residue and toward the $\alpha_1\beta_2$ interface. In addition the G helix and other regions of the α chain are also distorted (Greer, 1971).

Nmr studies of deoxy Hb Chesapeake show that the deoxy Hb Chesapeake structure differs somewhat from deoxy Hb A since the hyperfine shifted resonance at −7.9 ppm in the deoxy Hb A spectrum is missing in deoxy Hb Chesapeake and the other two proton resonances are shifted from their corresponding positions in Hb A. It has been shown that the hyperfine shifted resonances are very sensitive to heme–globin interactions and it is likely that the hyperfine shifted nmr spectra are reflecting differences in the deoxy structure in the region of the heme pockets of both the α and β chains between the structures of deoxy Hb A and deoxy Hb Chesapeake (Davis *et al.*, 1970, 1971).

In some hemoglobins nmr experiments have shown that in addition to being structurally nonequivalent, the α and β hemes are also functionally nonequivalent. Hb A in the presence of DPG or IHP exhibits a preferential decrease in the area under the α-heme peaks upon increasing oxygen saturation indicating that under equilibrium conditions oxygen binds to the α hemes in preference to the β hemes (Lindstrom and Ho, 1972; Johnson and Ho, 1974). Also in Hb A, it has been shown that *n*-butyl isocyanide binds preferentially to the β hemes in the presence of IHP (Lindstrom *et al.*, 1971). In Hb Kempsey [β99(G1) Asp→Asn] and Hb Yakima [β99(G1) Asp→His], CO is bound preferentially to the β hemes in the absence of organic phosphate or in the presence of DPG; but in the presence of IHP, CO binds randomly to the hemes (Ho *et al.*, 1973; Lindstrom *et al.*, 1973; K. J. Wiechelman and C. Ho unpublished results). Upon increasing CO saturation Hb Chesapeake is similar to Hb A in that CO appears to bind randomly to the α and β hemes. However, Hb Chesapeake differs from Hb A upon oxygenation in the presence of DPG by binding oxygen randomly while Hb A binds O_2 preferentially to the α hemes under similar conditions (Lindstrom and Ho, 1972; Johnson and Ho, 1974).

It is interesting to note that Nagel *et al.* (1967) have found that in both oxy and deoxy Hb Chesapeake the β93(F9) sulfhydryl groups are reactive toward iodoacetamide whereas in deoxy Hb A these groups are unreactive. In deoxy Hb A, histidine-β146(HC3) forms a loop around the F helix and its imidazole group forms a salt bridge with aspartate-β94 (FG1) of the same β chain and its α-carboxyl group makes a salt bridge with lysine-α40(C6) thus blocking access to the reactive SH groups (Perutz *et al.*, 1969). The β146 histidine to β94 aspartate salt bridge is responsible for about half of the alkaline Bohr effect in Hb A (Kilmartin and Wootton, 1970) and since Hb Chesapeake has a normal Bohr effect this salt bridge must be intact. Nmr studies of Hb Des-His 146β in which the β146 histidine residues have been removed enzymatically and of Hb Hiroshima [β146(HC3)His→Asp] (Perutz *et al.*, 1971) show that the binding of oxygen to the α and β chains is random even in the presence of IHP at pD 7 (Breen *et al.*, 1974; Lin, Breen, and Ho, unpublished results). These results may suggest that the conformation of the Hb molecule in the region affected by the β146 histidine salt bridges with β94 aspartate and lysine α40 may be critical for the ligand binding properties of the α and β hemes in a hemoglobin molecule. The fact that oxygen binding to Hb Chesapeake in the presence of DPG is random suggests that there may be some distortion in this region of the molecule. The results of our oxygen saturation studies in the presence of IHP suggest that the tighter binding of IHP to deoxy Hb Chesapeake may restore the conformation necessary for preferential binding of oxygen to the α hemes.

In a recent article (Ogata and McConnell, 1972), experimental results of a CO saturation study of Hb Chesapeake in the presence of the spin-labeled triphosphates, 1-oxyl-2,2,6,6-tetramethylpiperidine 4-triphosphate and N^6-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)adenosine triphosphate, were fit by a set of parameters calculated from a modified Monod, Wyman, and Changeux model (Monod *et al.*, 1965; Ogata and McConnell, 1971). Their results indicate that deoxy Hb Chesapeake contains molecules in the R (relaxed) as well as the T (tense) quaternary structures. If the rate of interconversion between the R and T structures is rapid on the nmr time scale, one would expect the resonances characteristic of each quaternary structure to coalesce into one broadened resonance with a chemical shift intermediate between the R and T chemical shifts. Perutz *et al.* (1974) have shown that the β-heme resonance which is found at ~−17.6 ppm in deoxy Hb A shifts upfield to ~−16 ppm in all deoxyhemoglobins having a R-like quaternary structure. In Figure 1 it can be seen that the β-heme resonance of deoxy Hb Chesapeake in 0.1 M Bis-Tris is broader than the β peak in deoxy Hb A under similar conditions and it has been shifted upfield from the β resonance of Hb A. It should be noted that the β-heme resonance of Hb Chesapeake in the absence of organic phosphate is broader than that of Hb Chesapeake and Hb A in the presence of DPG (Figure 1). This effect may be due to an equilibrium

between the R and T quaternary structures of Hb Chesapeake which would be consistent with the results of Ogata and McConnell (1972). A detailed discussion of T $\rightleftharpoons$ R in the $\alpha_1\beta_2$ mutants will be published elsewhere.

Our studies of the ring-current shifted proton resonances of the ligand-bound forms of Hb A and Hb Chesapeake show that nmr can be a powerful tool in investigations of structure–function relationships in hemoglobins (Lindstrom and Ho, 1973). The X-ray diffraction study of Hb Chesapeake (Greer, 1971) showed that the overall oxy Hb Chesapeake structure differs significantly from the oxy Hb A structure. However, the ring-current shifted proton resonances in the two hemoglobins show that they are very similar in the tertiary structures of the heme pockets. This result is consistent with the finding that k_4, the Adair constant for the binding of the fourth oxygen molecule to hemoglobin, is the same for Hb A and Hb Chesapeake (Imai, 1974).

A CO saturation study of Hb Chesapeake labeled with the spin-label *N*-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)iodoacetamide which binds to the β93(F9) sulfhydryl groups shows a sharp set of isosbestic points while the electron paramagnetic resonance (epr) spectra of Hb A labeled with the same iodoacetamide spin-label lack a set of isosbestic points upon CO saturation (Ho *et al.*, 1970). The presence of a set of isosbestic points in the epr spectra of the spin-labeled Hb Chesapeake on CO saturation has been suggested as evidence that there are differences in the $\alpha_1\beta_2$ subunit interactions between Hb A and Hb Chesapeake during CO saturation (Ho *et al.*, 1970; Baldassare *et al.*, 1970).

Our present results show that the structure of deoxy Hb Chesapeake is different from that of deoxy Hb A since the positions of the β-heme resonance as well as the position of the α-heme resonances have been altered. The ligand saturation studies of Hb Chesapeake also show differences from Hb A. The ring-current shifted proton resonances of Hb Chesapeake and Hb A indicate that the tertiary structures of the heme pockets of the ligand-bound forms of the hemoglobins are very similar. Thus, our nmr studies show that the initial state (deoxy) of Hb Chesapeake differs from the initial state of Hb A, but in the final state (oxy or carbonmonoxy) the tertiary structures of the heme pockets are the same for these two hemoglobins. Studies of the partially liganded forms of these hemoglobins show that the α and β chains in Hb Chesapeake are more equivalent in their reactions with O_2 and CO than the α and β chains in Hb A under similar conditions. Only in the case of oxygen saturation in the presence of inositol hexaphosphate do the two chains in Hb Chesapeake appear to be nonequivalent.

References

Antonini, E., Wyman, J., Brunori, M., Fronticelli, C., Bucci, E., Reichlin, M., and Rossi-Fanelli, A. (1964), *Arch. Biochem. Biophys. 108,* 569.

Baldassare, J. J., Charache, S., Jones, R. T., and Ho, C. (1970), *Biochemistry 9,* 4707.

Bartlett, G. R. (1950), *J. Biol. Chem. 234, 466.*

Berman, M., Benesch, R., and Benesch, R. E. (1971), *Arch. Biochem. Biophys. 145,* 236.

Breen, J. J., Bertoli, D. A., Dadok, J., and Ho, C. (1974), *Biophys. Chem. 2,* 49).

Dadok, J., Sprecher, R. F., and Bothner-by, A. A. (1972), Abstracts of the 13th Experimental NMR Conference, Pacific Grove, Calif.

Dadok, J., Sprecher, R. F., Bothner-by, A. A., and Link, T. (1970), Abstracts of the 11th Experimental NMR Conference, Pittsburgh, Pa.

Davis, D. G., Lindstrom, T. R., Mock, N. H., Baldassare, J. J., Charache, S., Jones, R. T., and Ho, C. (1971), *J. Mol. Biol. 60,* 101.

Davis, D. G., Mock, N. H., Lindstrom, T. R., Charache, S., and Ho, C. (1970), *Biochem. Biophys. Res. Commun. 40,* 343.

Glasoe, P. K., and Long, F. A. (1960), *J. Phys. Chem. 64,* 188.

Greer, J. (1971), *J. Mol. Biol. 62,* 241.

Hayashi, A., Suzuki, T., and Shen, M. (1973), *Biochim. Biophys. Acta 310,* 309.

Ho, C., Baldassare, J. J., and Charache, S. (1970), *Proc. Nat. Acad. Sci. U. S. 66,* 722.

Ho, C., Lindstrom, T. R., Baldassare, J. J., and Breen, J. J. (1973), *Ann. N. Y. Acad. Sci. 222,* 21.

Huisman, T. H. J., and Dozy, A. M. (1965), *J. Chromatogr. 19,* 160.

Imai, K. (1974), *J. Biol. Chem.* (in press).

Johnson, M. E., and Ho, C. (1974), *Biochemistry 13,* 3653.

Kilmartin, J. V., and Wootton, J. F. (1970), *Nature (London) 228,* 766.

Lindstrom, T. R., Baldassare, J. J., Bunn, H. F., and Ho, C. (1973), *Biochemistry 12,* 4212.

Lindstrom, T. R., and Ho, C. (1972), *Proc. Nat. Acad. Sci. U. S. 69,* 1707.

Lindstrom, T. R., and Ho, C. (1973), *Biochemistry 12,* 134.

Lindstrom, T. R., Ho, C., and Pisciotta, A. V. (1972a), *Nature (London), New Biol. 237,* 263.

Lindstrom, T. R., Norén, I. B. E., Charache, S., Lehmann, H., and Ho, C. (1972b), *Biochemistry 11,* 1677.

Lindstrom, T. R., Olson, J. S., Mock, N. H., Gibson, Q. H., and Ho, C. (1971), *Biochem. Biophys. Res. Commun. 45,* 22.

McDonald, C. C., and Phillips, W. D. (1967), *J. Amer. Chem. Soc. 89,* 6332.

McDonald, C. C., Phillips, W. D., and Vinogradov, S. N. (1969), *Biochem. Biophys. Res. Commun. 36* 442.

Monod, J., Wyman, J., and Changeux, J. P. (1965), *J. Mol. Biol. 12,* 88.

Nagel, R. L., Gibson, Q. H., and Charache, S. (1967), *Biochemistry 6,* 2395.

Ogata, R. T., and McConnell, H. M. (1971), *Cold Spring Harbor Symp. Quant. Biol. 36,* 325.

Ogata, R. T., and McConnell, H. M. (1972), *Proc. Nat. Acad. Sci. U. S. 69,* 335.

Ogawa, S., and Shulman, R. G. (1972), *J. Mol. Biol. 70,* 315.

Perutz, M. F. (1969), *Proc. Roy. Soc., Ser. B, 173,* 113.

Perutz, M. F. (1970), Nature (*London*) *228,* 726.

Perutz, M. F., Ladner, J. E., Simon, S. R., and Ho, C. (1974), *Biochemistry 13,* 2163.

Perutz, M. F., and Lehmann, H. (1968), *Nature (London) 219,* 902.

Perutz, M. F., Muirhead, H., Mazzarella, L., Crowther, R. A., Greer, J., and Kilmartin, J. V. (1969), *Nature (London) 222,* 1240.

Perutz, M. F., Pulsinelli, P. D., Ten Eyck, L., Kilmartin, J. V., Shibata, S., Iuchi, I., Miyaji, T., and Hamilton, H. B. (1971), *Nature (London), New Biol. 232,* 147.

Perutz, M. F., and Ten Eyck, L. (1971), *Cold Spring Harbor Symp. Quant. Biol. 36,* 295.

Shulman, R. G., Wüthrich, K., Yamane, T., Patel, D. J., and Blumberg, W. E. (1970), *J. Mol. Biol. 53,* 143.