Patel, D. J., Kampa, L., Shulman, R. G., Yamane, T., & Fujiwara, M. (1970) *Biochem. Biophys. Res. Commun. 40,* 1224.
Perutz, M. F., Ladner, J. E., Simon, S. R., & Ho, C. (1974) *Biochemistry 13,* 2163.
Shulman, R. G., Wüthrich, K., Yamane, T., Patel, D. J., & Blumberg, W. E. (1970) *J. Mol. Biol. 53,* 143.
Viggiano, G., Wiechelman, K. J., Chervenick, P. A., & Ho, C. (1978) *Biochemistry 17* (following paper in this issue).
Wiechelman, K. J., Charache, S., & Ho, C. (1974) *Biochemistry 13,* 4772.
Wiechelman, K. J., Fairbanks, V. F., & Ho, C. (1976) *Biochemistry 15,* 1414.

Proton Nuclear Magnetic Resonance Studies of Hemoglobins Osler (β145HC2 Tyr→Asp) and McKees Rocks (β145HC2 Tyr→Term): An Assignment for an Important Tertiary Structural Probe in Hemoglobin†

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ABSTRACT: High-resolution proton nuclear magnetic resonance studies of deoxyhemoglobins Osler (β145HC2 Tyr→Asp) and McKees Rocks (β145HC2 Tyr→Term) indicate that these hemoglobins are predominately in the oxy quaternary structure in 0.1 M [bis(2-hydroxyethyl)imino]-tris(hydroxymethyl)methane buffer at pH 7. Upon the addition of inositol hexaphosphate, the proton nuclear magnetic resonance spectra of these hemoglobins become similar to those characteristic of a hemoglobin molecule in the deoxy quaternary structure. The exchangeable proton resonance which is found at −6.4 ppm from H_2O in the spectrum of normal human adult deoxyhemoglobin is absent in the spectra of these two mutant hemoglobins. Consequently we believe the hydrogen bond between the hydroxyl group of tyrosine-β145HC2 and the carboxyl oxygen of valine-β98FG5 gives rise to this resonance. This assignment allows us to use the −6.4 ppm resonance as an important tertiary structural probe in the investigation of the cooperative oxygenation of hemoglobin.

Comparison of the high-resolution proton nuclear magnetic resonance (NMR)[1] spectra of proteins in H_2O and D_2O shows that exchangeable NH and/or OH protons give rise to resonances in the low-field region of the spectra (Glickson et al., 1969; Patel et al., 1970; McDonald et al., 1971; Ogawa et al., 1972, 1974; Ho et al., 1973, 1975; Mayer et al., 1973; Breen et al., 1974; Fung & Ho, 1975). ^1H NMR studies of hemoglobin in water have revealed several exchangeable proton resonances in the spectral region from ca. −9.4 to −5.5 ppm from H_2O (Patel et al., 1970; Ogawa et al., 1972, 1974; Ho et al., 1973, 1975; Mayer et al., 1973; Breen et al., 1974; Fung & Ho, 1975). The two exchangeable proton resonances which are found at −8.3 and −7.5 ppm from the H_2O resonance at 27 °C occur in the spectra of both liganded and unliganded hemoglobins. This suggests that they are located in a region of the hemoglobin molecule which is not significantly altered by the switch in quaternary structures, most likely in or near the $\alpha_1\beta_1$ subunit interface. The resonance at −8.3 ppm is believed to come from one of the hydrogen bonds formed by tyrosine-β35C1, probably the one to aspartate-α126H9 in the $\alpha_1\beta_1$ subunit interface (Asakura et al., 1976). The origin of the resonance at −7.5 ppm is not yet ascertained.

The presence of the remaining exchangeable proton resonances appears to depend on the quaternary structure of the hemoglobin molecule and can be used as markers for their respective quaternary structures. The resonances at −9.4 and −6.4 ppm from the proton resonance of H_2O in the spectrum of deoxy-Hb A are characteristic of the deoxy quaternary structure (Ogawa et al., 1972, 1974; Mayer et al., 1973; Ho et al., 1975; Fung & Ho, 1975). The resonance at −9.4 ppm has been assigned to the proton involved in the hydrogen bond between aspartic acid-β99G1 and tyrosine-α42C7, which stabilizes the $\alpha_1\beta_2$ subunit interface in the deoxy quaternary structure (Ho et al., 1975; Fung & Ho, 1975). The resonance at −5.5 ppm in the spectrum of HbCO A and at −5.8 ppm in HbO_2 A may come from the hydrogen bond between aspartic acid at α94G1 and asparagine-β102G4, which stabilizes the $\alpha_1\beta_2$ subunit interface in the oxy quaternary structure (Ho et al., 1975; Fung & Ho, 1975).

The hydrogen bonds between the penultimate tyrosine-HC2 and valine-FG5 of either the α or β chains have been suggested as possible candidates for the −6.4 ppm resonance (Fung &

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[1] Abbreviations used: Hb, Hemoglobin; Hb A, normal adult hemoglobin; HbCO, carbonmonoxyhemoglobin; HbO_2, oxyhemoglobin; P_2-glycerate, 2,3-diphosphoglycerate; Ins-P_6, inositol hexaphosphate; NMR, nuclear magnetic resonance; Tris, tris(hydroxymethyl)aminomethane; Bistris, [bis(2-hydroxyethyl)imino]tris(hydroxymethyl)methane; p_{50}, partial pressure of oxygen at 50% saturation; DSS, 2,2-dimethyl-2-silapentane-5-sulfonate; rf, radiofrequency.

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Ho, 1975). X-ray crystallographic studies of deoxyhemoglobin have shown that the penultimate tyrosines of both the α and β chains are firmly held in pockets between the F and H helices of the same chain (Perutz, 1970). The HC2 tyrosine is held in position partly through van der Waals interactions and partially by the hydrogen bond formed by its OH group and the carbonyl oxygen of valine-FG5. As ligands bind to the deoxyhemoglobin molecule, the F helix of a liganded subunit moves toward the center of the molecule. As the F helix moves toward the H helix, the pocket in which the penultimate tyrosine was held narrows and the tyrosine is expelled, causing the rupture of its hydrogen bond with valine-FG5. In oxyhemoglobin the penultimate tyrosines spend very little time in their pocket between the F and H helices (Perutz, 1970).

This communication reports our NMR studies of the mutant hemoglobins Olser[2] (β145HC2 Tyr→Asp) (Charache et al., 1975) and McKees Rocks[3] (β145HC2 Tyr→Term) (Winslow et al., 1976) which suggest that the −6.4 ppm exchangeable proton resonance arises from the hydrogen bond between the hydroxyl group of tyrosine-β145HC2 and the carbonyl oxygen of valine-β98FG5. The assignment of this tertiary structural probe along with the previous assignment of the −9.4 ppm resonance as a quaternary structural probe (Fung & Ho, 1975; Ho et al., 1975) should enable us to gain new insight into the molecular mechanism of oxygenation of both normal and abnormal human hemoglobins.

Experimental Section

Materials. Blood samples containing Hb Osler were kindly provided to us by Dr. Samuel Charache. Hb Osler was purified on DEAE-Sephadex (Pharmacia) using a linear pH gradient between pH 7.8 and 6.8 (S. Charache, personal communication). This is the same purification procedure used by Arnone et al. (1976) for Hb Nancy.[2] Blood samples from a heterozygous patient with Hb McKees Rocks were obtained locally. Hb McKees Rocks was purified on a column of CM-Sephadex (Pharmacia) using a linear pH gradient of 0.05 M Tris-maleate buffer from pH 6.7 to 7.0 (Winslow et al., 1976). Hb A was prepared in the usual manner from fresh whole blood obtained from the local blood bank (Lindstrom & Ho, 1972). Phosphate was removed from the hemoglobin samples by passing them through a column of Sephadex G-25 (Pharmacia) equilibrated with 0.01 M Tris buffer containing 0.1 M NaCl at pH 7.5 (Berman et al., 1971). The hemoglobin was then dialyzed exhaustively against deionized water to reduce the concentration of ions in the solution. Deoxyhemoglobin samples for ^{1}H NMR studies were prepared by standard procedures used in this laboratory (Lindstrom & Ho, 1972). All chemicals used were obtained from commercial suppliers and were used without further purification.

Methods. High-resolution ^{1}H NMR spectra at 250 MHz were obtained using the MPC-HF 250 MHz superconducting spectrometer (Dadok et al., 1970) at an ambient temperature of 27 °C. Chemical shifts are referenced to the water proton signal which is 4.83 ppm downfield from the proton resonance of 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) at 27 °C. A negative sign in the chemical shift indicates that the resonance is downfield from the water resonance. Signal-to-noise ratios were improved by signal averaging (about 1600 scans) using NMR correlation spectroscopy with a Sigma-5 computer interfaced to the MPC-HF 250 MHz spectrometer (Dadok & Sprecher, 1974). The sweep time for the spectral region from −1400 to −7400 Hz downfield from H_2O was 0.6 s. Double resonance experiments were performed by saturating the water proton resonance by a second radiofrequency (rf) pulse (Fung & Ho, 1975). The chemical shift measurements of these exchangeable proton resonances are accurate to ±0.1 ppm.

In order to get a better baseline for those exchangeable proton resonances close to the water proton resonance, we have used a higher frequency NMR spectrometer for some of our studies. The 360-MHz ^{1}H NMR spectra were obtained using a Bruker HX-360 NMR spectrometer located at the Stanford Magnetic Resonance Laboratory. The spectrometer was also operated on the correlation mode at a temperature of 25 °C. The sweep time for the spectral region from −1900 to −3600 Hz downfield from H_2O was 1 s with a delay of 0.3 s per scan for approximately 400 scans.

Results

The 250-MHz ^{1}H NMR spectrum of the exchangeable proton resonances of deoxy Hb A in 0.1 M Bistris at 27 °C contains resonances at −9.3, −8.2, −7.5, and −6.4 ppm from H_2O as shown in Figure 1A. A comparison of the relative intensities of the −6.4 and −9.4 ppm resonances made possible by the increased resolution at 360 MHz shows that these two resonances represent the same number of protons (i.e., two protons per hemoglobin tetramer) as shown in Figure 2. The addition of inositol hexaphosphate (Ins-P_6) to deoxy-Hb A causes no changes in its exchangeable proton resonances (Figure 2B). In the spectrum of deoxy Hb McKees Rocks, exchangeable proton resonances can be seen at −8.1, −7.5, and −6.2 ppm from H_2O in 0.1 M Bistris as shown in Figure 3A. There are also a number of less intense resonances at −9.3, −8.7, −6.9 and −6.5 ppm. The addition of 15 mM Ins-P_6 to deoxy Hb McKees Rocks results in an increase in the intensity of the resonance at −9.2 ppm; however, there is no apparent change in the −6.2-ppm resonance (Figure 3B). The small resonances observed in the spectrum in the absence of Ins-P_6 have disappeared.

In the 360-MHz ^{1}H NMR spectrum of deoxy-Hb Osler in 0.1 M Bistris, exchangeable proton resonances occur at −8.2 and −7.5 ppm as shown in Figure 4A. In addition, there is a broad resonance at about −6.3 ppm and a resonance of low intensity at ca. −9.2 ppm. The addition of 15 mM Ins-P_6 to Hb Osler results in a large increase in the intensity of the resonance at −9.2 ppm, while the resonance at −6.3 ppm in the absence of phosphate has sharpened and moved upfield slightly to −6.2 ppm as shown in Figure 4B.

The 250-MHz NMR spectra show that both the hyperfine shifted and exchangeable proton resonances of deoxy-Hb McKees Rocks and Hb Osler in 0.1 M Bistris differ from the corresponding resonances of deoxy-Hb A as shown in Figure 1. Hyperfine shifted resonances are located at −17.6, −12.4, and ca. −8 ppm in the spectrum of deoxy Hb A. The hyperfine shifted resonance at about −8 ppm is superimposed on the two exchangeable proton resonances at −8.2 and −7.5 ppm (Fung & Ho, 1975). The resonance at −17.6 ppm has been assigned to protons on the β chain and the resonances at −12.4 and −8 ppm come from α chain protons (Davis et al., 1971; Lindstrom et al., 1972; Ho et al., 1975; Fung et al., 1976, 1977). The addition of Ins-P_6 to deoxy-Hb A causes the β-heme resonance to shift downfield to ca. −18 ppm. The hyperfine shifted proton resonances of both deoxy-Hb Osler and Hb McKees Rocks in Bistris buffer are characteristic of those seen for deoxyhemo-

[2] Hemoglobins Nancy (Gacon et al., 1975) and Fort Gordon (Klecker et al., 1975) also have the mutation β145HC2 Tyr→Asp.

[3] Hemoglobin McKees Rocks has the same structure as carboxypeptidase A treated Hb A which is Des-Tyr β145-Des-His β146 and the two hemoglobins have identical functional properties (Winslow et al., 1976).

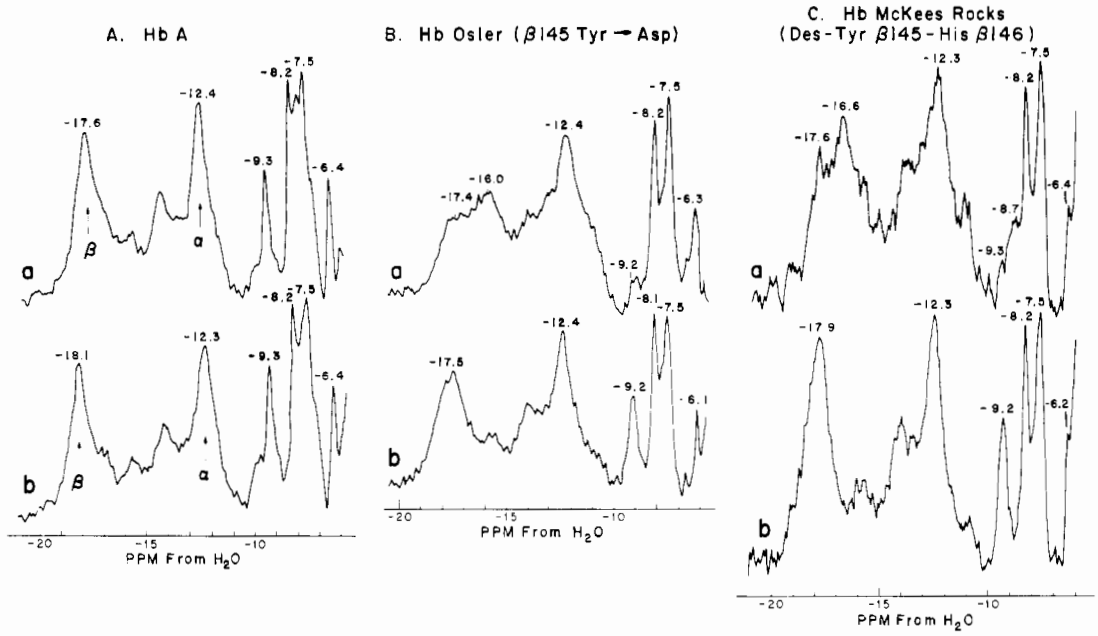

FIGURE 1: 250-MHz ^1H NMR spectra of the deoxyhemoglobins in H_2O at 27 °C in the spectral region containing the low-field exchangeable and hyperfine shifted proton resonances: Aa, 12.7% Hb A in 0.1 M Bistris at pH 6.8; Ab, the same as Aa but with 15 mM Ins-P_6 added; Ba, 12.7% Hb Osler in 0.1 M Bistris at pH 6.8; Bb, the same as Ba but in the presence of 15 mM Ins-P_6; Ca, 12% Hb McKees Rocks in 0.1 M Bistris, pH 7.0; Cb, the same as Ca but with 15 mM Ins-P_6 added.

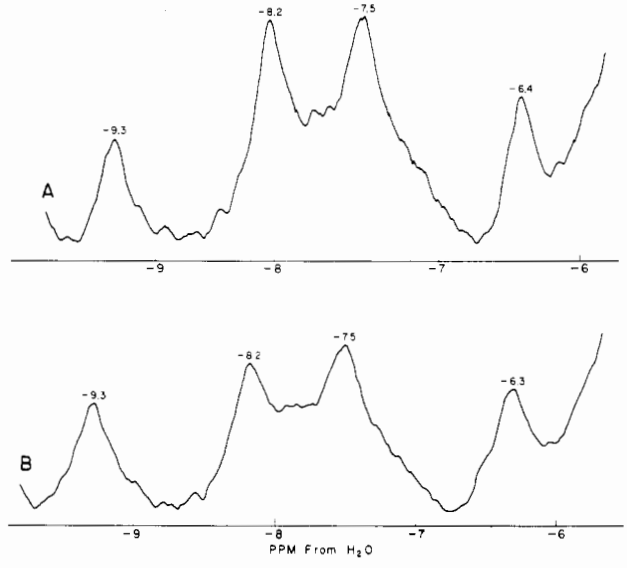

FIGURE 2: The 360-MHz ^1H NMR spectra of (A) 13.5% Hb A; and (B) 12% Hb A plus 10 mM Ins-P_6 both in 0.1 M Bistris in H_2O at pH 7.0 and 25 °C.

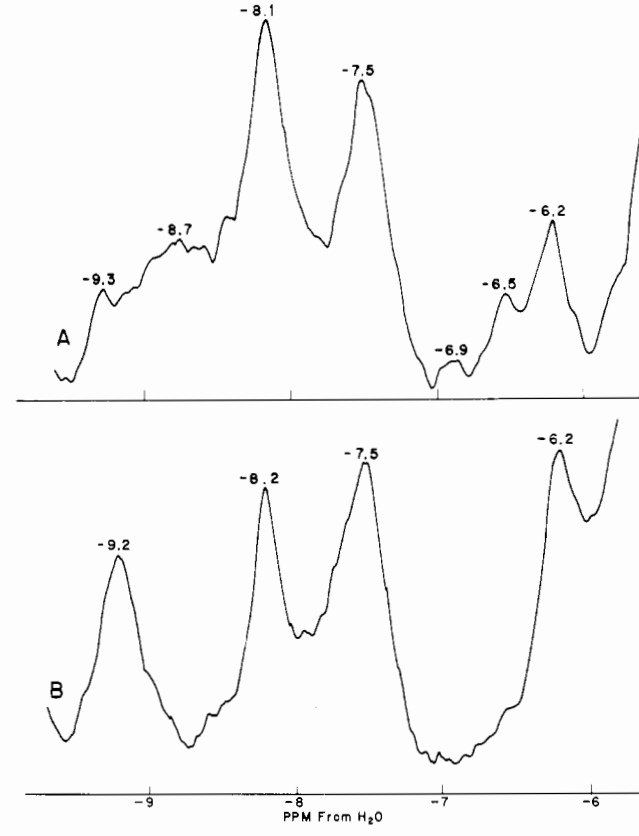

FIGURE 3: The 360-MHz ^1H NMR spectra of (A) 11.6% Hb McKees Rocks; and (B) 12.7% Hb McKees Rocks plus 15 mM Ins-P_6 both in 0.1 M Bistris in H_2O at pH 7.0 and 25 °C.

globins in the oxy quaternary structure (Ho et al., 1973; Perutz et al., 1974; Asakura et al., 1975; Weatherall et al., 1977). In the spectrum of deoxy Hb Osler (Figure 4A) there again appears to be a small resonance at −9.2 ppm, which may indicate that this hemoglobin contains a small amount of deoxy quaternary structure in equilibrium with the oxy quaternary structure. The addition of Ins-P_6 to unliganded Hb Osler and Hb McKees Rocks causes their hyperfine shifted proton resonances to become similar to those of deoxy-Hb A, indicating that they have switched to the deoxy quaternary structure. This is consistent with the increase in the intensity of the exchangeable proton resonances at −9.2 ppm in their spectra.

In order to investigate the nature of those resonances observed in deoxy-Hb McKees Rocks (Figure 3A) and Hb Osler (Figure 4A), two different types of experiments were performed. First, by saturating the water proton resonance with a second rf pulse, the intensities of exchangeable proton resonances should be greatly reduced (Fung & Ho, 1975). Figures 5A and 5B show clearly that the weak resonances observed in Figures 3A and 4A are indeed exchangeable protons because they disappear in the double resonance experiments. The broad

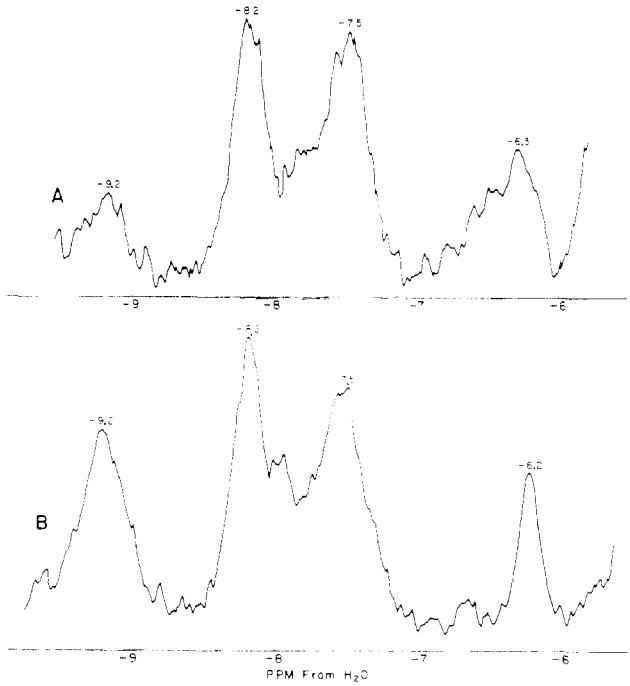

FIGURE 4: The 360-MHz ^1H NMR spectra of (A) 12.7% Hb Osler; and (B) 12.3% Hb Osler plus 15 mM Ins-P_6 both in 0.1 M Bistris in H_2O at pH 6.8 and 25 °C.

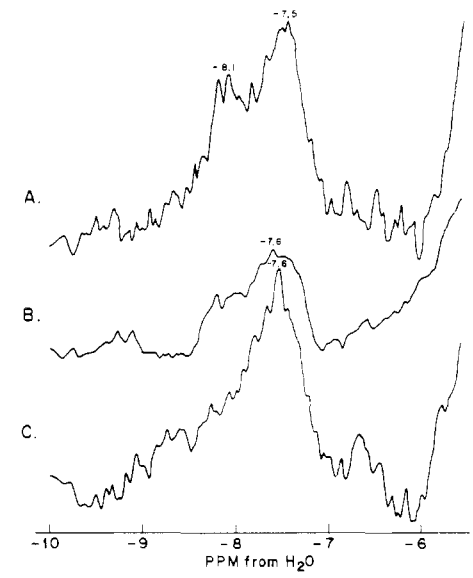

FIGURE 5: The 250-MHz ^1H NMR spectra of deoxyhemoglobins Osler and McKees Rocks in 0.1 M Bistris: (A) ~12% Hb Osler in H_2O at pH 6.8, with the water proton signal saturated by a second rf pulse in a double resonance experiment; (B) ~12% Hb McKees Rocks in H_2O at pH 6.8, with the water proton signal saturated by a second rf pulse in a double resonance experiment; (C) ~12% Hb McKees Rocks in 0.1 M Bistris plus 15 mM Ins-P_6 in D_2O at pD 7 (spectrum obtained without double resonance).

resonances at ca. −7.6 ppm in deoxy-Hb McKees Rocks (Figure 5A) and deoxy-Hb Osler (Figure 5B) arise from the hyperfine interaction between the unpaired electrons of Fe^{2+} and the proton groups in the porphyrin of the α chains in deoxyhemoglobin. Hence, this is a hyperfine shifted proton resonance. Figure 5C provides additional support to the conclusion reached in the above double resonance experiments. By obtaining the ^1H NMR spectrum of deoxy-Hb McKees Rocks in D_2O over the spectral region from −6 to −10 ppm, there is only one broad resonance at ca. −7.6 ppm (Figure 5C), characteristic of the hyperfine shifted proton resonance of the α chains in deoxyhemoglobin.

Discussion

The x-ray crystallographic studies of Perutz (1970) and Perutz & Ten Eyck (1971) have shown that there are a number of inter- and intrasubunit hydrogen bonds which help stabilize the deoxy quaternary structure of hemoglobin. These hydrogen bonds are formed between the following amino acids: tyrosine-α140HC2 and valine-α93FG5; tyrosine-β145HC2 and valine-β98FG5; histidine-β146HC3 and aspartic acid-β94FG1; arginine-α141HC3 and both lysine-α127H10 and aspartic acid-α126H9; lysine-α40C5 and histidine-β146HC3; and tyrosine-α42C7 and aspartic acid-β99G1. Earlier studies of the exchangeable proton resonances of the deoxy forms of Hb Des-Arg-α141 and Hb Des-His-β146 demonstrated that the −6.4 ppm resonance was present in the spectra of these hemoglobins (Fung & Ho, 1975). This finding suggests that hydrogen bonds involving arginine-α141HC3 and histidine-β146HC3 are not responsible for the −6.4 ppm resonances.

Our studies of Hb Osler and Hb McKees Rocks indicate that the −6.4 ppm resonance is missing in the spectra of these mutants and is not affected by the presence or absence of Ins-P_6. In addition, a new resonance is found at ca. −6.2 ppm. This exchangeable proton resonance does not appear in the spectrum of deoxy-Hb A and its origin is not known at this time. [This may be the same resonance which appears in the spectrum of deoxy-Hb NES-Des-Arg, Hb Kempsey, or Hb Yakima in the absence of Ins-P_6 (Fung & Ho, 1975)]. The decrease in the intensity of the −9.4 ppm resonance and the pattern of the hyperfine shifted resonances (a broad resonance ca. −16.5 ppm) suggest that unliganded Hb McKees Rocks and Hb Osler in 0.1 M Bistris are predominantly in the oxy quaternary structure. This is in agreement with the abnormally high oxygen affinities and lack of cooperative interactions in these hemoglobins (Nicklas et al., 1975; Arnone et al., 1976; Winslow et al., 1976; Bucci et al., 1978). Oxygen equilibrium measurements show that the addition of Ins-P_6 to Hb Nancy[2] results in an increase in its Hill coefficient from 1.1 to 2.0 and a decrease in its oxygen affinity ($\Delta \log p_{50} = 0.42$ at pH 7.2) (Arnone et al., 1976). The addition of Ins-P_6 to Hb McKees Rocks increases its Hill coefficient to 1.8 and decreases its oxygen affinity ($\Delta \log p_{50} = 0.61$) (Winslow et al., 1976). These findings are consistent with a change in the quaternary structure of these two mutant hemoglobins brought about by the addition of Ins-P_6 as manifested by the appearance of the quaternary structural probe at −9.2 ppm. This means that in the presence of Ins-P_6, deoxy-Hb McKees Rocks and Hb Osler have a deoxy-like quaternary structure. The position of this resonance in these two mutants is slightly different from that normally observed (i.e., −9.3 to −9.4 ppm) in deoxy-Hb A. This may suggest that the $\alpha_1\beta_2$ subunit interface is slightly distorted in these two abnormal hemoglobins. This is not surprising in view of the fact that (i) the mutation sites are not too far from the $\alpha_1\beta_2$ subunit interface and (ii) the functional properties of these two hemoglobins in the presence of Ins-P_6 are still different from Hb A.

Since the resonance at −6.2 ppm is present in the spectra of both the deoxy and oxy quaternary structures of unliganded Hb McKees Rocks and Hb Osler, it is not likely that this resonance is the deoxy tertiary structural marker observed in the spectrum of Hb A. It is possible that the −6.4 ppm resonance in Hb A has been shifted upfield by some structural perturbation in deoxy-Hb McKees Rocks and Hb Osler; for example, β98FG5 valine could form a hydrogen bond with another

residue in these two abnormal hemoglobins. Both hemoglobin Nancy[2] and Hb Des-Tyr β145-His-β146[3] form crystals which are in the deoxy quaternary structure (Perutz & Ten Eyck, 1971; Arnone et al., 1976). It is believed that the deoxy and oxy quaternary structures of unliganded Hb Des-Tyr-β145-His-β146 coexist in solution and crystallize in the deoxy quaternary structure since it is less soluble than the oxy quaternary structure (Perutz & Ten Eyck, 1971). This same effect may cause unliganded Hb Nancy[2] to form crystals which are in the deoxy quaternary structure. X-ray crystallographic studies of unliganded Hb Des-Tyr-β145-His-β146[3] at 3.5 Å resolution show that removal of the two carboxy terminal residues of Hb A cause a partial unwinding of the H helix as far back as alanine-β140H18, although the terminal dipeptide (lysine-β141HC1 and histidine-β143H21) is affected most (Perutz & Ten Eyck, 1971). X-ray studies of Hb Nancy[2] at 3.5 Å resolution show that the carboxy terminal tetrapeptide of the β chain is severely disordered, and as a result histidine-β146HC3 does not form either of its intrasubunit hydrogen bonds (Arnone et al., 1976).

In conclusion, we believe that our studies of the exchangeable proton resonances of Hb McKees Rocks and Hb Osler allow us to assign the exchangeable proton resonance at −6.4 ppm in the spectrum of deoxy-Hb A to the hydrogen bond between tyrosine-β145HC2 and valine-β98FG5. Our results also imply that the corresponding hydrogen bonds in the α chains occur in a different region of the ^{1}H NMR spectrum (i.e., they have a different environment from that in the β chains). The present assignment of the penultimate tyrosine of the β chain in a hemoglobin molecule provides us a unique opportunity to monitor the change in the tertiary structure in an important region of the molecule to that of the quaternary structural change as manifested by the resonance at −9.4 ppm.

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References

Arnone, A., Gacon, G., & Wajcman, H. (1976) *J. Biol. Chem. 251,* 5875.

Asakura, T., Adachi, K., Wiley, J. S., Fung, L. W.-M., Ho, C., Kilmartin, J. V., & Perutz, M. F. (1976) *J. Mol. Biol. 104,* 185.

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

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

Bucci, E., Charache, S., Nicklas, J., Weichelman, K., & Ho, C. (1978) manuscript in preparation.

Charache, S., Brimhall, B., & Jones, R. T. (1975) *Johns Hopkins Med. J. 136,* 132.

Dadok, J., & Sprecher, R. F. (1974) *J. Magn. Reson. 13,* 243.

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

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

Fung, L. W.-M., & Ho, C. (1975) *Biochemistry 14,* 2526.

Fung, L. W.-M., Minton, A. P., & Ho, C. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73,* 1581.

Fung, L. W.-M., Minton, A. P., Lindstrom, T. R., Pisciotta, A. V., & Ho, C. (1977) *Biochemistry 16,* 1452.

Gacon, G., Wajcman, H., Labie, D., & Vigneron, C. (1975) *FEBS Lett. 56,* 39.

Glickson, J. D., McDonald, C. C., & Phillips, W. D. (1969) *Biochem. Biophys. Res. Commun. 35,* 492.

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

Ho, C., Fung, L. W.-M., Weichelman, K. J., Pifat, G., & Johnson, M. E. (1975) in *Erythrocyte Structure and Function* (Brewer, G. J., Ed.) pp 43–64, Alan R. Liss, Inc., New York, N.Y.

Kleckner, H. B., Wilson, J. B., Lindeman, J. G., Stevens, P. D., Niazi, G., Hunter, E., Chen, C. J., & Huisman, T. H. J. (1975) *Biochim. Biophys. Acta 400,* 343.

Lindstrom, T. R., & Ho, C. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69,* 1707.

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

Mayer, A., Ogawa, S., Shulman, R. G., & Gersonde, K. (1973) *J. Mol. Biol. 81,* 187.

McDonald, C. C., Phillips, W. D., & Glickson, J. D. (1971) *J. Am. Chem. Soc. 93,* 235.

Nicklas, J., Charache, S., Wiechelman, K., Schaerf, S., Bucci, E., & Ho, C. (1975), Abstracts, the Eighteenth Annual Meeting of American Society of Hematology, Dallas, Texas, December 6–9, 1977.

Ogawa, S., Mayer, A., & Shulman, R. G. (1972) *Biochem, Biophys. Res. Commun. 49,* 1485.

Ogawa, S., Patel, D. J., & Simon, S. R. (1974) *Biochemistry 13,* 2001.

Patel, D. J., Kampa, L., Shulman, R. G., Yamane, T., & Fujiwara, M. (1970) *Biochem. Biophys. Res. Commun. 40,* 1224.

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

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

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

Weatherall, D. J., Cleggs, J. B., Callender, S. T., Wells, R. M. G., Gale, R. E., Huehus, E. R., Perutz, M. F., Viggiano, G., & Ho, C. (1977) *Br. J. Haemat. 35,* 177.

Winslow, R. M., Swenberg, M. L., Gross, E., Chervenick, P. A., Buchman, R. R., & Anderson, W. F. (1976) *J. Clin. Invest. 57,* 772.