Isotropically Shifted NMR Resonances for the Proximal Histidyl Imidazole NH Protons in Cobalt Hemoglobin and Iron–Cobalt Hybrid Hemoglobins. Binding of the Proximal Histidine toward Porphyrin Metal Ion in the Intermediate State of Cooperative Ligand Binding†

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ABSTRACT: Exchangeable imidazole NH proton nuclear magnetic resonance (NMR) signals of the proximal histidine F8, which is directly coordinated to the paramagnetic porphyrin cobalt ion, have been measured for cobalt-substituted deoxyhemoglobins (deoxy-CoHb) and iron–cobalt hybrid hemoglobins, $\alpha(Co)_2\beta(Fe)_2$ and $\alpha(Fe)_2\beta(Co)_2$. Comparison of NMR spectra between these Co-substituted hemoglobins and natural Fe-containing hemoglobin allows the assignment of the NH signals to the specific subunits, namely, a signal at 53.8 ppm to the α-subunits and a signal at 58.4 ppm to the β-subunits for deoxy-CoHb at 23 °C, respectively. The coordination of carbon monoxide to the β-subunits in $\alpha(Co)_2\beta(Fe)_2$ gave rise to an 8 ppm downfield shift of the proximal histidyl NH signal in the deoxy $\alpha(Co)_2$ subunits. In contrast, the coordination of carbon monoxide to the α-subunits in $\alpha(Fe)_2\beta(Co)_2$ showed only a 1 ppm downfield shift for the deoxy $\beta(Co)_2$ subunits. These observations suggest that the metal–histidyl coordination bonds in the deoxy cobalt subunits are strengthened by the ligation of carbon monoxide in the counterpart ferrous subunits. However, the change of the coordination was much larger in the $\alpha(Co)_2$ subunits than that in the $\beta(Co)_2$ subunits. Line-width analysis of the NH signals for CO-bound hybrid hemoglobins gave a mean exchange lifetime of 3.2×10^3 s^{-1} for the T to R quaternary structural transition. The proximal histidyl binding situation at some liganded states is discussed in relation to the quaternary structure of hemoglobin molecules.

The importance of the bond between the proximal histidine F8 imidazole and porhyrin metal ion in controlling the oxygen affinity of hemoglobin has been emphasized in a stereochemical mechanism (Perutz, 1970). In order to test this mechanism, numerous investigations have been conducted so far with chemical and physical methods (Perutz, 1979, 1980; Shulman et al., 1975). However, the most uncompromising problem that we encounter in studying the cooperative ligand binding to hemoglobin is the difficulty in directly investigating the intermediate state of ligand binding states during allosteric oxygen binding. For example, hyperfine-shifted resonances of deoxyhemoglobin have been examined for the monitoring of oxygen and carbon monoxide binding (Johnson & Ho, 1974; Huang & Redfield, 1976; Viggiano & Ho, 1979; Viggiano et al., 1979), but no physicochemical properties of the intermediate ligand binding states have been reported so far from such experiments. In this sense, iron–cobalt hybrid hemoglobins might be ideal models for testing not only the properties of the individual subunits within a tetramer but also those of the intermediate ligand binding states, because affinity of some small ligands toward Co subunits is so small compared with that toward Fe subunits (Ikeda-Saito et al., 1977). Especially, carbon monoxide cannot bind to the Co subunits in Fe–Co hybrid hemoglobins at atmospheric pressure, although it can strongly bind to the Fe subunits (Ikeda-Saito et al., 1977). Therefore, half-liganded hemoglobins, such as $\alpha(Co)_2\beta(Fe\cdot CO)_2$ and $\alpha(Fe\cdot CO)_2\beta(Co)_2$, can be easily prepared by flashing CO gas to the corresponding deoxy samples.

The exchangeable imidazole NH protons of the proximal histidine F8, which is directly coordinated to the paramagnetic porphyrin metal ion in deoxyhemoglobin, have been successfully detected and assigned to the specific subunits (La Mar et al., 1977, 1980; Takahashi et al., 1980). Owing to their large isotropic shifts caused by paramagnetism of the heme metal ion, these proton signals are expected to serve as potential markers in the investigation of the bonding between the proximal histidine and porphyrin metal. In this paper, we have observed imidazole NH signals of the proximal histidine for Co-substituted hemoglobins and compared NMR behavior of these proton signals in fully deoxyhemoglobins and half-liganded Fe–Co hybrid hemoglobins.

Experimental Procedures

Materials. Cobaltous protoporphyrin IX and cobalt-substituted hemoglobin (CoHb) were prepared after the literature methods (Yonetani et al., 1974). Iron–cobalt hybrid hemoglobins were prepared as reported previously (Ikeda-Saito et al., 1977). All other chemicals were from commercial sources and used without further purification.

Methods. Hemoglobin samples were deoxygenated by flashing argon gas and transferred under nitrogen atmosphere with a syringe to a spherical microcell (Wilmad 529-A), in which a small amount of solid sodium dithionite was placed in order to achieve complete deoxygenation. Immediately after the transfer of the hemoglobin solution, the stem of the microcell was sealed. We found that the hemoglobin thus prepared can be maintained in the deoxy state for more than 24 h.

Proton NMR spectra were obtained by a NIH 270 nuclear magnetic resonance (NMR) spectrometer that is composed of a Bruker superconducting magnet (6.35 T) and a Nicolet console equipped with a Nic 1180 computer system. The proton NMR signal is collected by a decoupler coil on a Bruker

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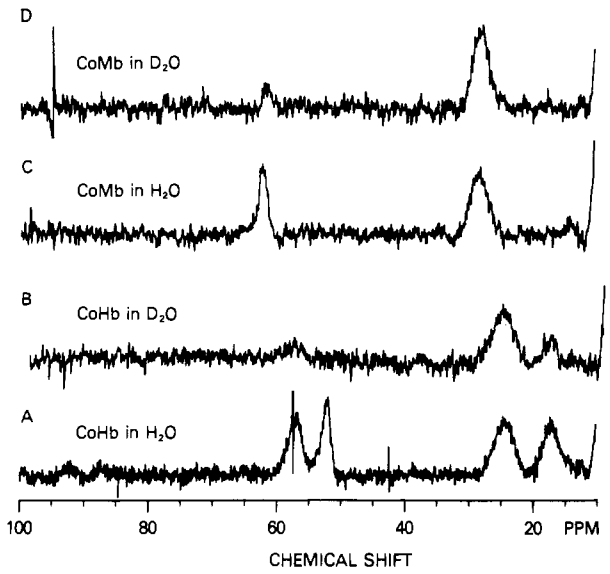

FIGURE 1: Proton NMR (270-MHz) spectra of deoxy-CoHb in (A) H_2O and (B) D_2O and of deoxy-CoMb in (C) H_2O and (D) D_2O. The CoHb sample was prepared in 50 mM tris(hydroxymethyl)-aminomethane (Tris) buffer at pH (or pD) 7.0, and CoMb was prepared in 0.1 M sodium phosphate at pH (or pD) 7.0. The signal at about 25 ppm for CoHb and the signal at 28 ppm for CoMb are nonexchangeable peaks, which may be assigned to meso protons of Co porphyrin (Hill et al., 1973). A trace of NH signal can still be observed after 3 h of D–H exchange, probably because of the slow exchange rate at 4 °C, where the sample was kept, and/or insufficient deuteration of buffer (about 90% deuterium enrichment).

5-mm ^{13}C probe. Typically, 11-μs (90°) radio-frequency pulses and ±30-kHz spectral width were applied to detect the proximal histidine NH signals for Co-substituted deoxyhemoglobins. Conventional WEFT pulse sequence (180°–τ–90°–acuire) was used in order to minimize the water signal. A careful setting of τ values (typically 70–100 ms) can completely eliminate the H_2O signal under rapid repetition of the sequence; regularly, an interpulse time of 0.1 s was applied. In order to obtain a reasonable signal to noise (S/N) ratio, 16–32 blocks of Fourier-transformed (FT) data after acquisition of 1K free induction decay (FID) signals were accumulated. Sensitivity of signals was artificially enhanced by multiplying by an exponential function, which produces Lorentzian line shape with 20-Hz additional line broadening. However, the exchange rate discussed in the following section was evaluated from the difference of two NMR line widths. Therefore, no artificial parameters are involved in the calculated rate. Chemical shifts were measured from the H_2O resonance position and converted to the shift from sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) by using a 4.8 ppm offset.

Results and Discussion

Figure 1A shows that deoxy-CoHb in H_2O solution exhibited two distinct hyperfine-shifted proton signals in extremely low field at 58.4 and 53.8 ppm. These two peaks were absent under the same pH and buffer conditions in D_2O solution (Figure 1B). Conversion to oxy-CoHb also gave rise to the disappearance of these exchangeable signals (data are not shown), indicating that these two signals are unique to deoxy-CoHb in H_2O solution. In addition, deoxy-CoMb (sperm whale) in H_2O solution showed a hyperfine-shifted signal at 62.2 ppm as shown in Figure 1C. This peak was also found to be absent in D_2O solution (Figure 1D). Such large paramagnetic shifts, which are substantially larger than the paramagnetic shifts of nonexchangeable proton signals observed in the high-field region (0 to ~−10 ppm) for Co-substituted deoxyhemoglobins (Ikeda-Saito et al., 1978), suggest that the protons responsible for these NMR signals must be located in close proximity to the CoHb paramagnetic center. So far, similar downfield paramagnetic shifts of more than 50 ppm were reported for deoxy-FeHb and -FeMb (La Mar et al., 1977, 1980; Takahashi et al., 1980). These signals have been assigned to the imidazole NH proton of the proximal histidine, which is directly coordinated to the paramagnetic heme iron. The above-mentioned observations together with other indirect evidence discussed later suggest that these large hyperfine-shifted proton signals of deoxy-CoHb and -CoMb may be assigned to the imidazole NH protons of the proximal histidine F8, which is directly bound to the paramagnetic porphyrin Co ion ($S = {}^1/_2$). Moreover, the two peaks observed for deoxy-CoHb are apparently due to the two different subunits of deoxy-CoHb.

Noteworthy is the 4.6 ppm separation of the two NH signals for deoxy-CoHb at 23 °C, which is significantly smaller than the separation of 13.1 ppm for FeHb reported by La Mar et al. (1980). Such a small difference in the resonance position may be adequately interpreted by assuming that the subunit inequivalence is significantly less in CoHb than in FeHb. In fact, the oxygen-binding analysis for CoHb showed a smaller difference in the oxygen affinity in the two subunits in CoHb than in FeHb (Imai et al., 1980).

The pseudocontact shift for the proximal histidyl NH proton was estimated with structural data of model complexes and anisotropic g values for the Co ion obtained in our previous study (Ikeda-Saito et al., 1977). The structural data for (1-MeIm)CoTPP (Scheidt, 1974), which has been considered as a relevant model complex of the R-state hemoglobin (Drago et al., 1977; Collman et al., 1978; White et al., 1979), give an upfield pseudocontact shift of 5.7 ppm at 20 °C. The data for (1,2-diMeIm)CoTPP (Dwyer et al., 1974), a model of the T-state hemoglobin (Drago et al., 1977; Collman et al., 1978; White et al., 1979), give an upfield pseudocontact shift of 5.5 ppm. Moreover, the diamagnetic contribution to the total NH shift was estimated to be upfield shift of approximately 3 ppm from the data for diamagnetic Ru(II)–porphyrin complexes (Satterlee & La Mar, 1976). Therefore, it is estimated that the downfield Fermi contact shift contributes more than 90% of the total isotropic shift. This large contribution is readily interpreted in terms of the direct spin transmission to imidazole from the d_{z^2} orbital of the Co ion where its unpaired spin ($S = {}^1/_2$) primarily resides in deoxy-CoHb and -CoMb.

In order to assign these signals to individual subunits of FeHb and CoHb, similar NMR measurements were carried out for Fe–Co hybrid deoxyhemoglobins in the presence and absence of CO. Figure 2B shows that deoxy $\alpha(Co)_2\beta(Fe)_2$ exhibited two isotropically shifted signals at 75.9 and 53.3 ppm, respectively. Comparison of the proton NMR spectra between $\alpha(Co)_2\beta(Fe)_2$ and CoHb shows that the signal that resonated at 58.4 ppm for CoHb is replaced by a signal at 75.9 ppm for $\alpha(Co)_2\beta(Fe)_2$, which was previously assigned to the imidazole NH proton of the proximal histidine in the $\beta(Fe)_2$ subunits for FeHb (La Mar et al., 1980; Takahashi et al., 1980). The signal at 53.8 ppm, which was observed in both CoHb and $\alpha(Co)_2\beta(Fe)_2$, can be eventually assigned to the NH of the proximal histidine in the $\alpha(Co)_2$ subunits. Therefore, the remaining signal at 58.4 ppm for CoHb can be identified as the NH of the $\beta(Co)_2$ subunits. Similar assignment was also carried out by comparison of the NMR spectra between $\alpha(Fe)_2\beta(Co)_2$ and CoHb, where a signal at 57.9 ppm is assigned to the NH in the $\beta(Co)_2$ subunits and a signal at 63.2 ppm

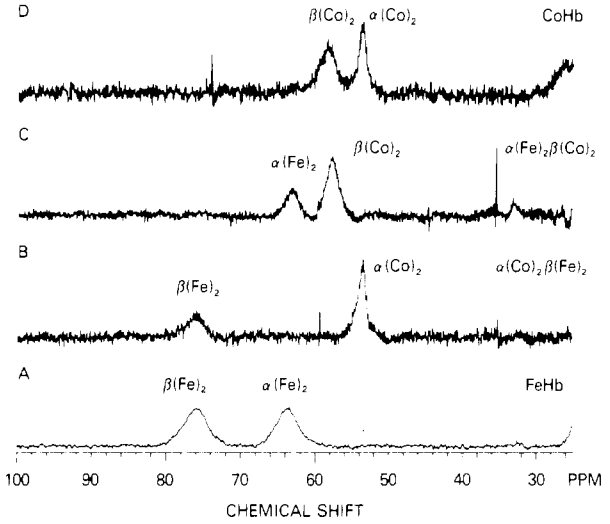

FIGURE 2: Comparison of proton NMR (270-MHz) spectra between FeHb and Co-substituted hemoglobins: (A) deoxy-FeHb; (B) deoxy $\alpha(Co)_2\beta(Fe)_2$; (C) deoxy $\alpha(Fe)_2\beta(Co)_2$; (D) deoxy-CoHb. All the samples were prepared in 50 mM Tris buffer at pH 7.0.

Table I: Resonance Positions of the F8 Histidine NH for Fe-Co Hybrid Hb's, CoHb, and FeHb and Their Assignments[a]

preparation	resonance position (ppm)			
CoHb	53.8	58.4		
$\alpha(Co)_2\beta(Fe)_2$	53.3			75.9
$\alpha(Fe)_2\beta(Co)_2$		57.9	63.2	
FeHb			63.2[b]	76.5[b]
$\alpha(Co)_2\beta(Fe{\cdot}CO)_2$	61.4			c
$\alpha(Fe{\cdot}CO)_2\beta(Co)_2$		58.7	c	
assignment	$\alpha(Co)_2$	$\beta(Co)_2$	$\alpha(Fe)_2$	$\beta(Fe)_2$

[a] Measured at 23 °C. [b] La Mar et al. (1980) and Takahashi et al. (1980). [c] $\alpha(Fe{\cdot}CO)_2$ and $\beta(Fe{\cdot}CO)_2$ subunits are diamagnetic and gave no paramagnetic shifts.

to the NH in the $\alpha(Fe)_2$ subunits. Again, such signal assignment may also indirectly support the preceding discussion that these peaks originate from the NH's of the proximal histidine. All the signal assignments and their resonance positions are assembled in Table I.

The most outstanding behavior of the NH signal in the Co subunits was observed as a dramatic 8.1 ppm downfield shift found in the deoxy $\alpha(Co)_2$ subunits in $\alpha(Co)_2\beta(Fe)_2$ upon CO coordination of CO to the $\beta(Fe)_2$ subunits as shown in Figure 3A,B. This signal also suffered significant line broadening, where the line width became 5-fold wider than that for the fully deoxy form. The NH signal in the β-subunits for $\alpha(Fe)_2\beta(Co)_2$ was also found to be shifted downfield by approximately 1 ppm upon CO binding to the counterpart $\alpha(Fe)_2$ subunits (Figure 3C,D). These shifts are enormously large when compared with the shift change observed by pH variation, because no appreciable shift was observed for these NH protons by pH change from pH 6.5 to pH 8.5 (data are not shown).

Straightforward interpretation of this line broadening for $\alpha(Co)_2\beta(Fe)_2$ is a line widening due to the increase in the electron-spin relaxation time (T_{1e}) of the porphyrin Co ion (La Mar et al., 1973). Generally, the longer the T_{1e} for the paramagnetic system, the broader the line of the isotropically shifted NMR signal. In fact, the corresponding EPR signal of $\alpha(Co)_2\beta(Fe{\cdot}CO)_2$ is significantly sharper than that of fully deoxy $\alpha(Co)_2\beta(Fe)_2$ at 77 K (Ikeda-Saito et al., 1977), indicating that the T_{1e} is relatively longer in the half-liganded state than in the fully deoxy state. In addition to this line-

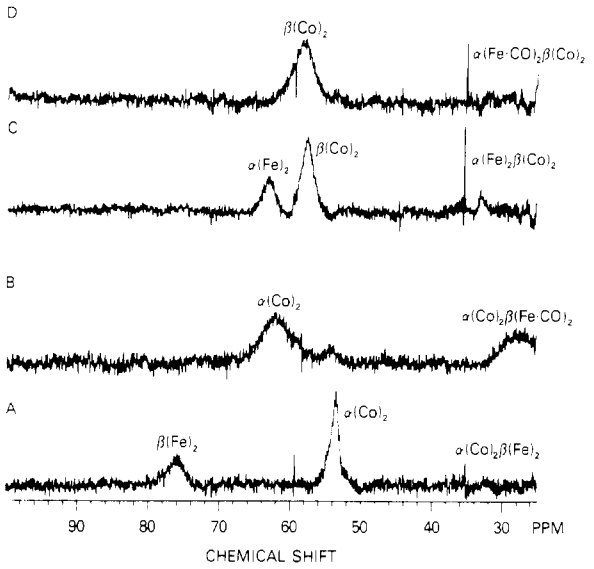

FIGURE 3: Comparison of proton NMR (270-MHz) spectra between fully deoxy and CO-saturated Fe–Co hybrid hemoglobins: (A) fully deoxy $\alpha(Co)_2\beta(Fe)_2$; (B) CO-saturated $\alpha(Co)_2\beta(Fe{\cdot}CO)_2$; (C) fully deoxy $\alpha(Fe)_2\beta(Co)_2$; (D) CO-saturated $\alpha(Fe{\cdot}CO)_2\beta(Co)_2$. All the samples were prepared in 50 mM Tris buffer at pH 7.0.

broadening mechanism, substantial increase of the line width was also observed in the NH signal for the $\beta(Co)_2$ subunits in $\alpha(Fe{\cdot}CO)_2\beta(Co)_2$, where the corresponding EPR spectra did not show an essential change in its line width, namely, T_{1e}, by CO ligation (Ikeda-Saito et al., 1977). This observation suggests that the signals in the half-liganded states may suffer additional line broadening other than the broadening due to the increase of T_{1e}, probably exchange line broadening. However, the off-rate of the CO ligand from the coordination site of the Fe subunits has been reported to be too slow, 0.012 s^{-1} at 23.5 °C and pH 9.1 (Gibson & Roughton, 1957), to explain this exchange line broadening. Therefore, the line broadening by CO-ligand exchange can be excluded from consideration. The previous study of oxygen binding for Fe–Co hybrid hemoglobins (Ikeda-Saito & Yonetani, 1980) showed relatively higher oxygen affinity of the deoxy Co subunits in the half-liganded forms when compared with that for the corresponding fully deoxy states. It was concluded from these observations that their quaternary structures in the half-liganded states inclined somewhat to the R state even though the Co subunits are in the unliganded state. Therefore, the T- to R-state interconversion, which may modulate the conformation of protein and/or the proximal histidine binding to the heme metal, may be responsible for the above exchange line broadening. In the case of $\alpha(Fe{\cdot}CO)_2\beta(Co)_2$, where the change of T_{1e} is not expected from its EPR spectrum, the exchange line broadening of the NH signal was observed as approximately 1 kHz, which gives 3.2×10^3 s^{-1} for a mean exchange rate. So far, Ogawa et al. (1974) have reported the lower limit of the R to T conversion rate, 10^4 s^{-1}, for des-Arg-Hb by the analysis of hyperfine-shifted NMR signals.[1] This value is in good agreement with present results.

[1] Very recently, Nagai et al. (1982) estimated the interconversion rate as 8×10^2 s^{-1} on the basis of the line-broadening analysis of the proximal histidyl NH signal for des-Arg-Hb (H. F. Bunn, personal communication). However, the present analysis shows that the T–R-state interconversion rate is faster for Co-substituted Hb than that for des-Arg-Hb. Such discrepancy may partly be caused by the difference in the liganded status of measured hemoglobins, where the rate for Co-substituted Hb was measured in the half-liganded state but that for FeHb was in fully deoxy state.

A large downfield shift of the *proximal* histidyl NH signals in the Co subunits upon coordination of CO to the counterpart Fe subunits may provide some interesting information on the quarternary structural change of hemoglobin during cooperative ligand binding. As discussed earlier in this paper, the paramagnetic shifts originate primarily from the Fermi contact shift, which is directly proportional to the spin density induced on the protons. The further increases of the downfield Fermi contact shift of 8 ppm for the $\alpha(Co)_2$ subunits and of 1 ppm for the $\beta(Co)_2$ subunits indicate that the spin density on the NH proton propagated through the cobalt–histidyl bond is increased upon ligation of CO to the partner Fe subunits. It can be inferred further that CO ligation to the Fe subunits gives rise to a strengthening of the proximal histidine binding to the porphyrin Co ions in the partner deoxy Co subunits. Moreover, the substantially larger downfield shift observed for the α-subunits than for the β-subunits indicates that such a change in the metal–histidyl bond in the α-subunits is more pronounced than in the β-subunits.

Such interpretations are in good agreement with those obtained from the corresponding electron paramagnetic resonance (EPR) and resonance Raman studies. Previous EPR study (Ikeda-Saito et al., 1977) for Fe–Co hybrid hemoglobins showed that the spectrum for deoxy $\alpha(Co)_2$ subunits was more remarkably influenced in its line shape and position upon ligation of CO than that of deoxy $\beta(Co)_2$ subunits. Recent resonance Raman studies for valency hybrid hemoglobins (Nagai & Kitagawa, 1980) and for Fe–Co hybrid hemoglobins (Ondrias et al., 1982) also have shown that the band due to the stretching of the bond between the *proximal* histidyl imidazole nitrogen and porphyrin metal is shifted more profoundly for the α-subunits by the T–R transition of hemoglobin than for the β-subunits. However, these histidyl NH proton signals were observed to be insensitive to pH change, whereas the EPR spectra of the deoxy Co subunits in these compounds were sensitive to both pH changes and the ligation of CO to the partner Fe subunits (Ikeda-Saito et al., 1977). Therefore, it is reasonable to suggest that the electronic state of the Co ion is modulated by both pH and the ligation to the partner subunits, whereas the metal–histidyl coordination bond is not sensitive to the former but influenced by the latter.

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References

Collman, J. P., Brauman, J. I., Doxsee, K. M., Halbert, T. R., Hayes, S. E., & Suslick, K. S. (1978) *J. Am. Chem. Soc. 100*, 2761–2766.

Drago, R. S., Beugelsdijk, T., Breese, J. A., & Cannady, J. P. (1977) *J. Am. Chem. Soc. 100*, 5374–5382.

Dwyer, P. N., Madura, P., & Scheidt, W. R. (1974) *J. Am. Chem. Soc. 96*, 4815–4819.

Gibson, Q. H., & Roughton, F. J. W. (1957) *Proc. R. Soc. London, Ser. B 147*, 44–56.

Hill, H. O. A., Sadler, J. P., & Williams, R. J. P. (1973) *J. Chem. Soc. A*, 1663–1667.

Huang, T.-H., & Redfield, A. G (1976) *J. Biol. Chem. 251*, 7114–7119.

Ikeda-Saito, M., & Yonetani, T. (1980) *J. Mol. Biol. 138*, 845–858.

Ikeda-Saito, M., Yamamoto, H., & Yonetani, T. (1977) *J. Biol. Chem. 252*, 8639–8644.

Ikeda-Saito, M., Inubushi, T., McDonald, G. C., & Yonetani, T. (1978) *J. Biol. Chem. 253*, 7134–7137.

Imai, K., Ikeda-Saito, M., Yamamoto, H., & Yonetani, T. (1980) *J. Mol. Biol. 138*, 635–648.

Johnson, M. E., & Ho, C. (1974) *Biochemistry 13*, 3653–3661.

La Mar, G. N., Horrocks, W. D., & Holm, R. H., Eds. (1973) *NMR of Paramagnetic Molecules*, Academic Press, New York.

La Mar, G. N., Budd, D. L., & Goff, H. (1977) *Biochem. Biophys. Res. Commun. 77*, 104–110.

La Mar, G. N., Nagai, K., Jue, T., Budd, D. L., Gersonde, K., Sick, H., Kagimoto, T., Hayashi, A., & Taketa, F. (1980) *Biochem. Biophys. Res. Commun. 96*, 1172–1177.

Nagai, K., & Kitagawa, T. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 2033–2037.

Nagai, K., La Mar, G. N., Jue, T., & Bunn, H. F. (1982) *Biochemistry 21*, 842–847.

Ogawa, S., Patel, D. J., & Simon, S. R. (1974) *Biochemistry 13*, 2001–2006.

Ondrias, M. R., Rousseau, D. L., Kitagawa, T., Ikeda-Saito, M., Inubushi, T., & Yonetani, T. (1982) *J. Biol. Chem. 257*, 8766–8770.

Perutz, M. F. (1970) *Nature (London) 228*, 726–739.

Perutz, M. F. (1979) *Annu. Rev. Biochem. 48*, 327–386.

Perutz, M. F. (1980) *Proc. R. Soc. London, Ser. B 208*, 135–162.

Satterlee, J. D., & La Mar, G. N. (1976) *J. Am. Chem. Soc. 98*, 2804–2808.

Scheidt, W. R. (1974) *J. Am. Chem. Soc. 96*, 90–94.

Shulman, R. G., Hopfield, J. J., & Ogawa, S. (1975) *Q. Rev. Biophys. 8*, 325–420.

Takahashi, S., Lin, A. K.-A. C., & Ho, C. (1980) *Biochemistry 19*, 5196–5202.

Viggiano, G., & Ho, C. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 3673–3677.

Viggiano, G., Ho, N. T., & Ho, C. (1979) *Biochemistry 18*, 5238–5247.

White, D. K., Cannon, J. B., & Traylor, T. G. (1979) *J. Am. Chem. Soc. 101*, 2443–2454.

Yonetani, T., Yamamoto, H., & Woodrow, G. V., III (1974) *J. Biol. Chem. 249*, 682–690.