Structural Changes in Ferric Chain on Oxygenation of Ferrous Chain in Hemoglobin Milwaukee†

K. C. Mishra, Santosh K. Mishra, C. P. Scholes, and T. P. Das*

Contribution from the Department of Physics, State University of New York, Albany, New York 12222. *Received April 19, 1984*

Abstract: Electron nuclear double-resonance measurements of hemoglobin Milwaukee have shown that there is a 2% increase in the ^{14}N hyperfine constant for the nitrogen ligand (N_ϵ) of iron belonging to the proximal histidine in the β-chain on oxygenation of the α-chain. In this paper, we have demonstrated, through electronic structure investigations, that this change can be ascribed to a contraction of the Fe–N_ϵ bond by 0.026 Å. This result provides independent support for the bond extension model proposed in the literature to explain the changes in Fe–N_ϵ stretching frequencies for the α- and β-chains in deoxyhemoglobin, which are associated with the R → T transition and are observed through resonance Raman spectroscopy measurements. The energetics of the bond length alteration in hemoglobin Milwaukee are discussed, and comparisons are made with the corresponding quantities in deoxyhemoglobin.

The structural changes in hemoglobin systems associated with the T → R transition have been, and will continue to be, a subject of great interest in understanding the process of oxygenation of these systems. Recent Raman resonance spectroscopy measurements[1] have provided evidence for a weakening of the Fe–N_ϵ bond in going from R to T state, the infrared frequency for the α-chain changing from 223 to 203 cm^{-1} and for the β-chain from 224 to 217 cm^{-1} in a set of hemoglobin systems. As explained below, two possible mechanisms have been proposed for this observed weakening of the Fe–N_ϵ bond. One involves[1a] an extension of the Fe–N_ϵ bond related to the tension model[2] of the cooperativity. The second[3] is a covalent mechanism involving the influence on the Fe–N_ϵ bond of the change in hydrogen bonding of the H attached to the N atom of imidazole. This mechanism is related to the proposition[4] in the literature that the N_δ–H bond may play a role in influencing oxygen affinity. In the present work we have demonstrated that the interpretation of data from an entirely independent source, namely, the ^{14}N hyperfine interaction studied by electron nuclear double-resonance (ENDOR) measurements[5] in hemoglobin Milwaukee with oxygenated and deoxygenated α-chains, provides support for the bond length alteration mechanism.

In the bond-extension model, the Fe–N_ϵ bond has been ascribed[1a] an increase of 0.024 Å for the α-chain and 0.008 Å for the β-chain by using a Morse potential function analysis, the strain energies being about 31 and 4 cal/mol, respectively in going from R to T state. The second, or covalent, model was based on results of nuclear magnetic resonance measurements,[6] where the change in the chemical shift of the exchangeable hydrogen attached to N_δ was ascribed to a change in the hydrogen bonding of this hydrogen with the protein chain. It has been proposed[3] that this change in hydrogen bonding could induce an alteration in the Fe–N_ϵ covalent bonding, which could provide an alternate interpretation of the resonance Raman data. However, an examination[7] of the relationship of the changes in the proton chemical shift and the Fe–N_ϵ stretching frequency in a number of mutant hemoglobins has shown that no simple correlation exists between the two properties. This could be considered to suggest that the covalent mechanism,[3] while it may be present, is not sufficient to provide the necessary weakening of the Fe–N_ϵ bond strength for explaining the observed change in the Fe–N_ϵ stretching frequency.

With the ENDOR measurements, the results of which are of interest to us in the present work, it was found[5] that on oxygenation of the ferrous α-chains of Hb Milwaukee, an increase of 2% occurred in the hyperfine constant for the $^{14}N_\epsilon$ nucleus of the proximal histidine in the ferric β-chain. While the Fe–N_ϵ bonds in the deoxyHb and met β-chains of Hb Milwaukee are expected to be different in strength, since they involve ferrous and ferric states, respectively, of the iron, the met system does undergo quaternary changes[8] in methemoglobin when an R → T transition occurs under the influence of IHP. One could therefore consider any change in the Fe–N_ϵ bond length for the β-chain in Hb Milwaukee on oxygenation of the α-chain to be analogus in nature, though different in strength, to that in deoxyHb as one goes from the T state to the R state. Our present work shows that the alteration in the $^{14}N_\epsilon$ hyperfine constant for the β-chain on oxygenation of the α-chain in hemoglobin Milwaukee can be explained through a contraction of the Fe–N_ϵ bond length, the direction of change being the same as derived[1a] from resonance Raman data, since the transition occurring in the present case is a T → R one.

Procedure, Results, and Discussion

The procedure utilized for our analysis of the change in $^{14}N_\epsilon$ hyperfine constant is similar to that employed in our earlier investigations[9] of high- and low-spin heme systems. The most recent investigation[9e] pertinent to the present work was carried out in metmyoglobin where experimentally observed differences[10] in hyperfine interactions among porphyrin ^{14}N nuclei in single-crystal metmyoglobin were interpreted in terms of Fe–N bond distances associated with the pyrrole ligands. In the recent single-crystal ENDOR work on metmyoglobin,[10] it has been concluded that the major parts of the ^{14}N hyperfine interaction constants for the porphyrin and proximal histidine nitrogen (N_ϵ) arise from the Fermi contact interaction. We have, therefore, as in our earlier work[9e] on metmyoglobin, assumed that the change in $^{14}N_\epsilon$ hy-

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perfine interaction for the β-chain, on oxygenation of the α-chain, can also be ascribed primarily to the isotropic contact interaction term A_i. In terms of the molecular orbitals for the heme unit, consisting of the iron, porphyrin ring, and the fifth and sixth ligands, A_i is given by[9e]

$$A_i = \frac{8\pi}{6S}\gamma_e\gamma_n\hbar^2|\psi_{2s}(0)|^2\sum|C_{\mu,2s}|^2 \quad (1)$$

where $S = {}^5/_2$ is the total spin, γ_e and γ_n are the gyromagnetic ratios for the electron and ^{14}N nucleus, $|\psi_{2s}(0)|^2$ is the density at the ^{14}N nucleus due to a single 2s electron, and $C_{\mu,2s}$ is the coefficient of the nitrogen 2s orbital in the μth molecular orbital, the summation in μ running over all the five unpaired orbitals in the system. In the present work, we have used the electronic wave functions of aquometmyoglobin as representative of the wave functions of the β-chain in Hb Milwaukee, since these wave functions were available from our earlier work.[9e] Actually, in Hb Milwaukee, the sixth ligand of iron in the β-chain is the carboxyl group[11] of the glutamic acid that replaces the valine E11(67)β, instead of the water molecule in aquometMb. The Fe in Hb Milwaukee appears from X-ray data[11] to be bonded to an oxygen of the carboxyl group making the nature of the bond between iron and the sixth ligand similar to that in the aquomet system. It thus seems reasonable to use the latter system as the model for our investigations of the $^{14}N_\epsilon$ hyperfine interactions and its changes[5] in Hb Milwaukee.

The variation in A_i in eq 1 arises from the changes in $|C_{\mu,2s}|^2$. These changes were studied by carrying out, as in our earlier work,[9e] self-consistent charge extended Hückel calculations for a number of Fe–N_ϵ distances in the range ± 0.25 Å of the equilibrium distance R_0 for aquometMb. In the course of these investigations, the Fe–N distances for the pyrroles of the porphyrin ring were kept constant as suggested by EXAFS measurements[12] for the environment of Fe in deoxy- and oxyhemoglobins. The variations $\delta(A_i)$ in A_i obtained by using eq 1 were fitted to an expression of the form

$$\delta(A_i)/A_i = 1 + \alpha\Delta + \beta\Delta^2 \quad (2)$$

Δ being the change in angstroms from in the Fe–N_ϵ bond distance from R_0. The power series form in eq 2 is based on the Taylor expansion and is justified because of the rapid convergence that was found in powers of Δ.

From the fit in eq 2, the experimental ENDOR result of 2% change in $^{14}N_\epsilon$ hyperfine constant for the β-chain on oxygenation of the α-chain led to $\Delta = -0.026$ Å, that is a contraction. This contraction is of the same order of magnitude and in the same direction as obtained[1] from the resonance Raman measurements of the Fe–N_ϵ stretching frequency in deoxyHb in going from the T to R state, the transition expected in the β-chain on oxygenation of the α-chain. Our result for the Fe–N_ϵ bond contraction is a factor of 3 higher than that derived[1] from the infrared frequency shift using a Morse approximation to the potential governing the Fe–N_ϵ vibrational motion. A part of this difference could be due to the use[1] of the Morse approximation, but perhaps the major source of this difference is the fact that in Hb Milwaukee, the Fe–N_ϵ bond involves the ferric state of iron, while in deoxyHb, one deals with the ferrous state. The important point is that our interpretation of the ENDOR hyperfine shift data[5] in Hb Milwaukee supports the direction and the order of magnitude of the change in Fe–N_ϵ bond length derived from resonance Raman data. It thus lends support to the strain model[1] for the infrared frequency change for the Fe–N_ϵ stretching vibration in going from the T to R state.

Further, by a parabolic fit of the total energies from our calculation for the three points corresponding to the equilibrium Fe–N_ϵ distance and distances larger and smaller than this by 0.025 Å, we have obtained a force constant $K = 110$ kcal/(mol/Å^{-2}). When one combines this with the change of 0.026 Å in the Fe–N_ϵ distance derived from the ENDOR data, this leads to a change in energy for the Fe–N_ϵ bond of 37 cal/mol for the ferric β-chain on oxygenation of the α-chain. These results are to be compared with the values[13] of K of about 115 kcal/mol for both ferrous α- and β-chains in deoxyHb, changes[1a] in Fe-N_ϵ bond length for the T $\rightarrow$ R transition of 0.024 and 0.008 Å, respectively, and energy changes of 31 and 4 cal/mol. The remarkable similarity of the force constants K for the ferric and ferrous chains is not too surprising because the Fe and N atoms were found in our work[9] to carry relatively small charges showing that the formal charge of 3+ on iron in the ferric compounds is distributed through the entire heme unit involving iron and its six ligands. Thus, the change in formal charge on iron in going from the ferrous to ferric compound is not expected to influence the Fe–N_ϵ bonding drastically. It is interesting, however, that the bond extension in going from R $\rightarrow$ T state for the ferric β-chain in Hb-Milwaukee is about a factor of 3 larger than for[1a] the ferrous β-chain in deoxyHb. It will be interesting to examine the sources for this difference, a likely cause being the possibility of different heme–protein interactions in the two cases. Lastly, the 37 cal/mol energy change for the Fe–N_ϵ bond in the ferric β-chain in going from the T to R state, on oxygenation of the α-chain, is a very small percentage of the 3.4 kcal/mol estimated[14] to be associated with the increase in oxygen-binding affinity between the first and last oxygen molecule to the Hb tetramer. Thus, our analysis shows that only a small fraction of the total energy associated with the T to R conformational change is stored at the ferric heme site in the β-chain. This is in keeping with the proposal[15] that the major part of the conformational energy change is communicated to weaker bonds throughout the protein chains.

Registry No. oxy Hb Milwaukee, 37294-38-5; Hb Milwaukee, 9035-05-6; heme, 14875-96-8; oxygen, 7782-44-7.

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