# Stereoelectronic Control on the Coordination of Substrates to Globin Proteins. The Role of Proximal His93 on the NO Release from Myoglobin

Dóra K. Menyhárd[†] and György M. Keserű*[,†,‡]

*Department of Theoretical Chemistry*
*Eötvös Loránd University*
*P.O. Box 32, H-1112 Budapest, Hungary*
*Department of Chemical Information Technology*
*Technical University of Budapest*
*P.O. Box 91, H-1521 Budapest, Hungary*

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Although the protein−ligand complexes of hemoglobin (Hb) and myoglobin (Mb) have been extensively studied,[1] it is still in debate how these proteins differentiate between physiological $O_2$, poisonous CO,[2] or redox active NO ligand and how the binding and release of these ligands is modulated by protein conformational motions. Mutation experiments,[3] IR[4] studies, and ab initio calculations[5] of $MbFe^{II}$−CO showed that the nature and exact conformation of the proximal amino acid connecting to the heme affects the binding geometry and affinity of the ligand.

NO is also a biologically significant, but less studied, ligand of the globin protein family. NO is unique in the sense that it can always bind to the heme of these proteins regardless of the oxidation state of the iron, and is even responsible for the interconversion of these different redox states. It can be both the oxidizing[8] and reducing agent[8] of the $Hb/MbFe^{II} \leftrightarrow Hb/MbFe^{III}$ cycle and therefore has a special regulating effect. It can also protect from possible oxidative damages caused by the formation of $Hb/MbFe^{IV}{=}O$ species by reducing this form to $Hb/MbFe^{III}$.[10] The $MbFe^{III}$−NO complex is especially intriguing since from it either dissociation and reassociation of NO or—at higher concentrations—reduction of the $Fe^{III}$ heme center may take place.[7]

We studied this complex to see if, similar to $MbFe^{II}$−CO, the proximal amino acid's influence could be detected and computationally confirmed. An indicative picture could be gained by studying the crystal structures available in the Protein Data Bank[10,11] (PDB) as to the general behavior of the proximal amino acid His93 (proxHis) of Mb. In a series of structures presenting the results of mutation experiments aimed at studying the significance of the distal His64 (distHis) of Mb,[12] in some cases, the water molecule—regularly the sixth ligand around the iron—was absent (Table 1). The most striking conformational change coupled to the presence or absence of this sixth ligand was the flip of the CA−CB−CG−CD2 torsion of proxHis. The significance of this is emphasized by recent $^1$H NMR[13] and resonance Raman investigations of the distal pocket mutants that suggest the presence or absence of water affects the heme iron−proxHis bonding character.[14]

**Table 1.** Coordination of the Crystallographic Water Molecule in Mutant (HOH155) and Wild Type (HOH156) Myoglobin PDB Structures

| name of PDB entry | distal amino acid | water in 6th position | torsion angle of proxHis |
|---|---|---|---|
| pdb2mgj.ent | Val | absent | −124.7° |
| pdb2mge.ent | Leu | absent | −122.0° |
| pdb2mgi.ent | Thr | partially occupied | −119.3° |
| pdb2mgh.ent | Gln | present | −113.5° |
| pdb2mgb.ent | Gly | present | −114.5° |
| pdb1ymb.ent[16] | His (wild type) | present | −113.8° |

Here we present the results of calculations considering different states of this torsion angle, between −113° (which corresponds to the value found in the not mutated, wild type, structure with water as the sixth ligand) and −125° (ligand free state). The X-ray structure of the $MbFe^{III}$−NO complex has not been determined yet, so we built our model using the coordinates of $MbFe^{III}$.[15] This active site model contains the iron, the heme, and all residues in close contact, Phe43, His64, Val68, Leu89, His93, and His97, a total of 193 atoms (Figure 1). NO was placed 1.96 Å from the iron in a bent conformation, on the basis of small molecular crystallographic results for a model heme compound[16] and the structure of the $HbFe^{III}$−NO[17] complex. The NO complex of a similar $Fe^{III}$ heme protein with coordinated proximal histidine was also refined with a bent $Fe^{III}$NO conformation.[18]

Due to the relatively large active site model, its electronic structure was calculated at the INDO/S CI semiempirical level,[19] in a coordinate system centered on the iron. NO runs parallel to the *x* axis in the *xz* plane in the positive, while proxHis is in the negative *x* direction from Fe. Calculated Soret maxima and Q-bands were in good agreement with the experimental data[8] (418, 536, and 570 nm and 415, 543, and 577 nm, respectively) for the wild type structure, revealing that our calculations provide a fair electronic description of these systems. On the basis of IR measurements, Rella et al.[4] proposed that protein motions, including the conformational changes of the proximal amino acid, produce fluctuating electric fields which give rise to time-dependent back-bonding. According to their model, the heme serves as an antenna that communicates these conformational variations to the ligand. Back-bonding interactions have been established as key factors in the stabilization of other heme protein−substrate complexes as well, such as cytochrome P450,

[†] Eötvös Loránd University.
[‡] Technical University of Budapest.

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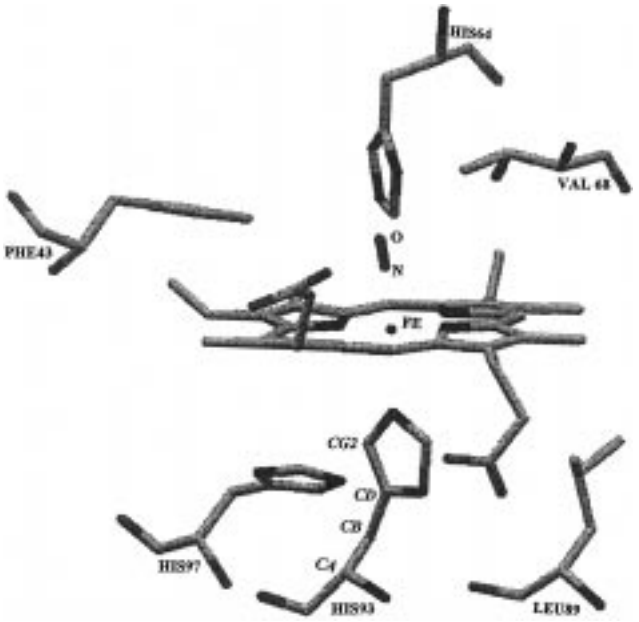


**Figure 1.** Active site model of myoglobin applied.

**Table 2.** Atomic Orbital Coefficients of the Back-Bonding MO at Different Orientations of the ProxHis Residue and Relative Energies (kJ/mol) of the Corresponding NO Complex and NO Free States

| AO coefficient at | torsion angle of proxHis | | | | | | |
|---|---|---|---|---|---|---|---|
| | −113° | −115° | −117° | −119° | −121° | −123° | −125° |
| Fe $3d_{z^2}$ | −0.044 | −0.043 | −0.041 | −0.039 | −0.036 | −0.032 | −0.026 |
| Fe $3d_{x^2-y^2}$ | −0.068 | −0.066 | −0.065 | −0.063 | −0.059 | −0.054 | −0.047 |
| Fe $3d_{xy}$ | −0.213 | −0.211 | −0.208 | −0.202 | −0.193 | −0.177 | −0.154 |
| Fe $3d_{xz}$ | 0.045 | 0.045 | 0.044 | 0.043 | 0.041 | 0.037 | 0.032 |
| Fe $3d_{yz}$ | −0.055 | −0.057 | −0.059 | −0.060 | −0.061 | −0.061 | −0.058 |
| N of NO $2p_x$ | −0.013 | −0.014 | −0.017 | −0.021 | −0.024 | −0.029 | −0.033 |
| N of NO $2p_y$ | −0.258 | −0.254 | −0.248 | −0.239 | −0.225 | −0.204 | −0.174 |
| proxHis NE2 $2p_x$ | −0.069 | −0.084 | −0.100 | −0.117 | −0.137 | −0.158 | −0.177 |
| proxHis NE2 $2p_y$ | −0.130 | −0.131 | −0.133 | −0.134 | −0.134 | −0.131 | −0.124 |
| relative energy of the NO complex | 0 | −2.34 | −3.86 | −4.88 | −4.63 | −3.86 | −1.97 |
| relative energy of the NO free state | 0 | −2.87 | −4.96 | −6.59 | −7.13 | −6.99 | −5.56 |

NOS, and peroxidases; therefore, their influence on the substrate binding of globin proteins is not unexpected.

Since the relative orientation of the coordinated ligands has a strong influence on back-bonding, we proposed that conformational changes of the proximal ligand alone should have a characteristic effect on these interactions. Back-bonding is characterized as a $\pi$-symmetry interaction between similarly oriented d and p atomic orbitals of the central iron and the ligated nitrogens, respectively. The dominating back-bonding MO, the highest MO with considerable coefficient on the iron, was here found to be HOMO − 7, 0.05 eV below the HOMO level. As the torsional angle of proxHis was varied from −113° to −125° by 2° steps, the MO coefficients of the nitrogen of the NO, Fe, and NE2 were compared (Table 2). We found a distinct transition in these values when changing the torsional angle from − 113° to −125°.

The results show that modification of the torsion angle of the proximal ligand, a quite subtle conformational change, considerably rearranges the bonding interactions between the proxHis and the heme, and between the heme and the NO ligand. Since NO lies in the *xz* plane, $\pi$ interaction between its N and the Fe is the result of the mixing of $2p_y$ and $3d_{xy}$ orbitals. The His N $2p_y$ AO also overlaps with the $3d_{xy}$ AO of Fe but in an antibonding manner. Therefore, the $\pi$ interaction between NO and Fe becomes gradually weaker as the torsional angle of proxHis is varied from −113° to −125°; simultaneously the His−Fe $\pi$ interaction becomes stronger, i.e., less antibonding in character. $\sigma$ bonding created by the mixing of $2p_x$ orbitals of the nitrogens with the $3d_{x^2-y^2}$ of iron follows the same course. His becomes more $\sigma$ bound to Fe, while antibonding between Fe and NO becomes stronger. As can be seen, the twist of the proximal ligand results in weakened back-bonding to NO; however, bonding interaction between the proximal ligand and the iron is made more effective.

**Table 3.** Electrostatic Atomic Charges at the Different Orientations of the ProxHis Residue

| charge at | torsion of proxHis | | charge at | torsion of proxHis | |
|---|---|---|---|---|---|
| | −113° | −125° | | −113° | −125° |
| Fe | 1.49 | 1.38 | O of NO | 0.17 | 0.16 |
| NE2 of proxHis | −0.19 | −0.05 | NE2 of distHis | −0.21 | −0.26 |
| N of NO | −0.37 | −0.37 | | | |

The relative energy of the NO complex goes through a potential well along the flip of proxHis in the middle of the torsion angle range, but the difference in energy between the −113° and −125° states (1.97 kJ/mol) is practically within the computational error of the applied method. However, the stablilty of the corresponding NO free state increases as the torsion is flipped from −113° to −125° (by 5.56 kJ/mol), demonstrating that the conformational motion of proxHis has an effect on the NO free heme.

To see if the heme really had an antenna type effect, we repeated the calculations, excluding the porphyrin from the model. Changing the torsion angle of proxHis from −113° to −125° in this case resulted in virtually unaltered MO distribution, supporting the notion that the ring system around the iron has a vital role in the communication between the proximal residue and the ligand. If we, therefore, view the heme as an antenna, the role of the proximal amino acid might be that of the *tuner* which selects between the ligated and the unligated forms.

Electrostatic atomic charges for the two conformational states obtained by UHF/STO-3G[20] calculations were also compared (Table 3). It is apparent that besides the back-bonding effect, electrostatic attraction between the iron and NO also decreases upon the flip of proxHis. Altering charges of the heme atoms create a new electrostatic environment for the ligand. It was interesting to see that modest changes of the charges of distal side amino acids, especially that of NE2 of distHis, can also be seen.

Calculations confirm that the back-bonding interaction between the iron and the ligand is strongly influenced even by slight conformational changes of the protein. Results suggest that the experimentally observed strengthening of proxHis−Fe covalency upon the loss of a water molecule as the sixth ligand can be explained by the coupled twist of the proxHis torsion. We propose this motion to be the dominating protein conformational change inititating the release of the NO ligand. Back-bonding interactions were identified as the most important stereoelectronic factors responsible for this action.

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