The Proximal Residue Largely Determines the CO Distortion in Carbonmonoxy Globin Proteins. An ab Initio Study of a Heme Prosthetic Unit

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How the globin proteins execute their physiological role of oxygen transport and storage in the presence of significant CO concentrations has been the focus of considerable research interest.[1] The physiological ligand, O_2, binds to free porphyrin in a bent conformation,[2] which, in the globin protein, is further stabilized by a hydrogen bond to the terminal oxygen atom.[3] However, the poisoning ligand, CO, binds to free porphyrin perpendicular to the heme plane,[2] up to 25 000 times more strongly than oxygen.[4] This is reduced to a ratio of only 30 in myoglobin,[1] and how the heme pocket discriminates in favor of oxygen has become an important example of the control exerted by a protein over its binding site (see, for example, ref 4).

The first high-resolution X-ray structure of MbCO showed the Fe−C−O unit bent and tilted away from the distal side chain, the nearest residue to the binding site (Protein Data Bank[5,6] (PDB) structure 1MBC[7]). These conformations were interpreted in terms of a steric repulsion between the distal residue and the CO ligand,[7−9] forcing the carbonyl into a nonperpendicular binding geometry and thereby reducing its affinity for the protein binding site. However, the bend angles observed in the crystal structures are large, and it has been difficult to account for such large strain energies being delivered by the distal side chain,[9] which, being exposed to the solvent, is quite mobile, as confirmed by the X-ray *B* factors[7] and in molecular dynamics simulations.[10,11] More recently, following a series of experimental studies of site-specific mutant MbCO proteins, it has been proposed that the key distal residue−CO interaction is electrostatic in nature: only those distal mutants with a polar side chain show any significant change in their infrared (IR) spectra from that of the His64Gly mutant.[12] Little difference was found between the IR[12] and resonance Raman[13] spectra of distal mutants with aliphatic side chains of various steric bulk, nor did their X-ray structures differ significantly.[14] It has therefore been proposed that the true orientation of the Fe−C−O unit in the protein is nearer the perpendicular,[15,16] and that the Fe−C−O bonding is largely determined by the extent of electrostatic polarization by the nearby distal residue.[12,16,17] It has also been suggested that the geometries proposed in the original X-ray structures were subject to significant experimental error.[16] However, if this electrostatic interpretation of the distal−CO interaction is correct, then the distal−CO interaction is *attractive*, i.e., the distal residue is *stabilizing* the CO ligand and cannot be responsible for the large changes in the relative affinity of CO and O_2 for the heme when located inside the protein.

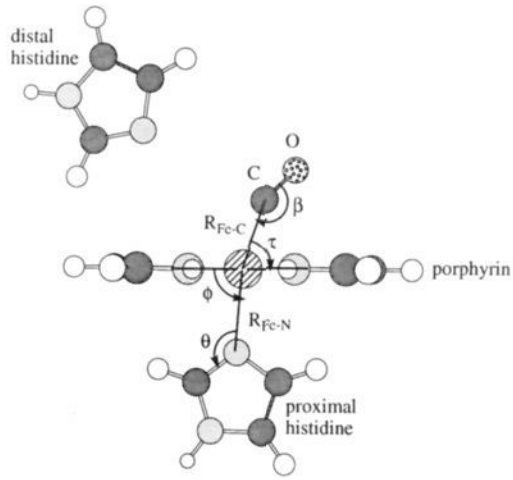

Figure 1. Model heme prosthetic group based on the X-ray structure PDB file 1MBC. $R_{Fe-N} = 2.19$ Å, $\phi = 86.3°$, and $\theta = 130.3°$.

Here we communicate the preliminary results of an *ab initio* investigation of the CO orientation in the heme pockets of globin proteins. These results suggest that the distorted binding, i.e., destabilization, of CO in the protein is largely determined by the nonequilibrium orientation of the proximal histidine; the geometrical influence of the distal residue is small in comparison.

In the present calculations, the porphyrin macrocycle was simplified to two amidinato ligands[18,19] and the distal and proximal residues to their imidazole side chains. The locations of the distal and proximal imidazole rings were determined from the X-ray structure (PDB file 1MBC[7]) but rotated to maintain C_s symmetry (Figure 1). A Hay and Wadt 16-electron pseudopotential[20] was used at the Fe center, with Goddard's triple-ζ d orbital contraction.[21] The axial ligands were described by

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Table 1. MP2 Optimization of the CO Orientation in a Model Heme Prosthetic Group Based on the PDB Structure 1MBC, Both with and without the Distal Residue (Upper), and the Orientation Determined Experimentally in the PDB Structure (Lower)[a]

		structural parameters			
		R_{Fe-C}/Å	tilt[b] τ/deg	bend, β/deg	CO orientation[c]
Ab Initio Optimization					
proximal only		1.79	75.85	159.44	34.7
distal and proximal		1.91	72.39	151.73	45.9
PDB Structure					
1MBC[d]	C	1.92	89.2	141.4	39.4
	D	1.92	89.2	119.9	59.6

[a] A perpendicular Fe−C−O geometry, $\beta = 179.6°$, $\tau = 88.6°$, and $R_{Fe-C} = 1.79$ Å, was found at the MP2 level when the orientation of the proximal residue was allowed to relax from that in the protein. This equilibrium orientation of the proximal residue has $\phi = 90.4°$, $\theta = 126.7°$, and $R_{Fe-N} = 1.92$ Å. [b] Orientation of the Fe−C bond relative to the mean plane through the four porphyrin nitrogen atoms. An angle of 90° lies on the heme normal. [c] Orientation of the C−O bond relative to the normal to the mean plane through the four porphyrin nitrogens. An angle of 0° lies on the normal. [d] Two carbonyl O positions were resolved: C with occupancy 78% and D with occupancy 22%.

Dunning's (9*s*/5*p*) bases,[22] contracted to [6111/411/1] with additional polarization and diffuse functions included. The equatorial ligands were described by minimal STO-3G bases[23] and the distal residue by 3-21G bases.[24] All geometry optimizations were constrained to C_s symmetry and calculated at the MP2 level using Gaussian92.[25] The bend (β), tilt (τ), and Fe−C bond length (R_{Fe-C}) have been optimised, both in the presence and in the absence of the distal residue, with the positions of all the other centers determined from the X-ray structure. The C−O bond length (R_{C-O}) was kept frozen at 1.13 Å, consistent with the free carbon monoxide bond length[26] and the bond length found in the model heme compound, Fe^{II}(TPP)(pyr)(CO),[27] to prevent the overestimate of Fe−C backbonding found in test calculations. The results, together with the Fe−C−O geometry found in the X-ray structure, are summarized in Table 1. Further calculations showed that the angle θ is the key determinant of the Fe−C−O distortion in this model system. In these supplementary calculations, the orientation of the proximal imidazole ring was varied over the range of angles observed in the X-ray structures of MbCO (1MBC and 2MB5) and carbonmonoxy hemoglobin (1HCO, 1COH, 1SDH, 1ECO, and 2HCO), and the orientation of the CO group was then reoptimized at the SCF level. The ϕ and θ angles were independently varied between 80° and 90° in steps of 2.5° and between 110° and 135° in steps of 5°, respectively; all other proximal parameters were fixed to their average values in the X-ray structures. Over these ranges, the Fe−C−O geometry was left almost unaltered by the changes in ϕ (changes of less than 1.5° in the CO orientation relative to the heme normal), whereas with θ greater than 125° or less than 115°, a large change in the CO orientation of up to 30° was found.

It is clear from Table 1 that the nonequilibrium orientation of the proximal residue is largely responsible for the nonperpendicular orientation of the CO ligand in this model heme prosthetic unit. Including the distal residue in the optimization has a smaller influence over the final Fe−C−O geometry. The distortion of the CO ligand in the supermolecular *ab initio* calculation is similar to that found in the MbCO X-ray structure, 1MBC. Thus, if the same mechanism is applicable to the protein, then the large strain energies implied by the large Fe−C−O distortion found in the X-ray structure are delivered by the protein tertiary structure *via the proximal residue and not by the mobile distal side chain.* That is, the proximal residue is largely responsible for discouraging the binding of CO to the protein active site, not the distal side chain. This is consistent with the geometry adopted by the proximal ligand when severed from the protein tertiary structure[28] and with the nonperpendicular Fe−C−O geometry found even in the His64Gly mutant.[14] Furthermore, this result reconciles the orientation of the distal side chain found in the X-ray structures with the largely geometry-innocent influence of the distal residue over the Fe−C−O bonding, as inferred from the IR spectra of the mutant MbCOs in solution.[12] The distal side chain is not in a sterically crowded position since the proximal residue causes the CO to bind off the heme normal; thus, the position of the distal side chain in the solvated protein can be determined by a weak attractive electrostatic interaction with the carbonyl group.[11,12] The failure of recent molecular dynamics simulations to find any significant distortion of the Fe−C−O group from the perpendicular,[11,29] while demonstrating that there was little systematic steric distortion of the Fe−C−O geometry by the distal side chain, could therefore be an artifact of neither force field allowing for the coupling of the heme axial ligand motions through the Fe *d* orbitals.

The combination of these theoretical results with the other experimental results that have become available recently leads to a fundamental reassessment of the structure−function relationship in one of the best known protein families; a picture is emerging whereby the Fe−C−O distortion and electron distribution are largely determined by the proximal residue but fine-tuned by electrostatic interactions with the distal residue.

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Supplementary Material Available: Investigation of the effect of basis set quality and the simplified porphyrin macrocycle on the orientation of the CO ligand (4 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered for the ACS; see any current masthead page for ordering information.

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