Ab initio study on the molecular recognition by metalloporphyrins: CO interaction with iron porphyrin

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We have performed *ab initio* pseudopotential calculations to study the effects of structural deformations of iron porphyrin on the configuration of a carbon monoxide (CO) attached to it. We have considered two proximal deformations around the heme group: (i) rotation of a pyrrole ring in the iron porphyrin, and (ii) rotation of the imidazole side chain bound to the iron atom. We have identified induced changes of the atomic geometry and the electronic structure of the iron porphyrin–CO complex, and the results elucidate the microscopic nature of the CO interaction with the iron porphyrin. Implications on the controversies over the binding angle of the CO molecule on the iron porphyrin under different circumstances are discussed. A potential application to the simulation-based chemical sensor design is also discussed. [S1063-651X(99)10002-3]

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I. INTRODUCTION

A metalloporphyrin is a planar molecule with one metal atom chelated at the center of a porphyrin macrocycle, as sketched in Figs. 1(a) and 1(b), and this metal atom plays a central role in molecular recognition and chemical selectivity of the metalloporphyrin. Since the chemical reactivities for various ligands are greatly modified by the central metal atom [1], metalloporphyrins are also used in the design of state-of-the-art chemical sensors such as an electronic nose and an electronic tongue, i.e., gas and liquid phase molecular sensors [2]. The best known example of metalloporphyrins in biomolecular systems is probably the iron porphyrin (so called a heme group) in myoglobin or hemoglobin. In spite of the diversity of the metalloporphyrins in molecular recognition applications, the detailed selection mechanism is not completely understood yet.

The famous puzzle over the ligand binding in myoglobin and hemoglobin is a notable example. The role of myoglobin is to store oxygen in human muscles while hemoglobin with a similar structure carries oxygen from the lung along the blood vessel. The iron atom at the center of the heme group is coordinated with five nitrogen atoms, four in the porphyrin cycle and one in the imidazole ring from the His63 side chain. Small molecules like O_2, CO, and NO bind to the empty sixth site above the iron atom, as shown in Fig. 2. The binding affinity of CO to a pentacoordinated *model* heme is 30 000 to 100 000 times greater than that of O_2. However, this ratio is reduced to 30 to 200 when the heme is embedded in the protein matrix [3]. The underlying mechanism of the ligand discrimination in myoglobin or hemoglobin has attracted a lot of attention from theorists and experimentalists. Understanding the mechanism is also important for the practical application of manufacturing artificial blood [4].

It has been a central dogma in the field that the bent geometry of CO observed in x-ray crystallography [5] is the major cause of the affinity reduction of CO. [6] However, this idea was challenged recently by sophisticated infrared experiments which observe very tiny bending angles [7,8]. The steric repulsion from the His64 was believed to be the main driving force for bending the CO molecule, but many site-directed mutagenesis studies do not show a consistent result with this belief [9]. Recently, Jewsbury *et al.* [10] have

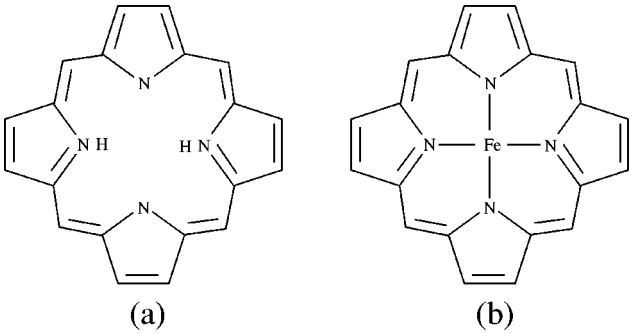

FIG. 1. Schematic structures (top down view) of the (a) porphyrin and (b) iron porphyrin.

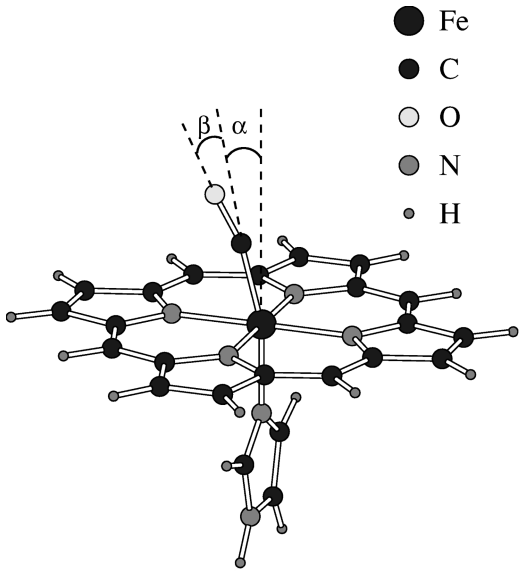

FIG. 2. The heme model system considered in the calculation. For visual clarity, the rotation angle of CO is exaggerated.

proposed that the orientation of the proximal imidazole is a major factor in determining the CO geometry. This proposition was contradicted by a recent calculation using the density functional method with an accurate basis set [11].

Despite several theoretical works, a detailed analysis on the effects of the interaction between the heme group and surrounding ligands is yet to be performed. In this paper, we carry out *ab initio* calculations for the effect of the distortion around the heme group on the binding geometry of the CO molecule. We consider proximal distortions around the iron atom that differentiate the geometry of the heme group from that of the model system. We have analyzed the protein databank (PDB) parameters for several structures of CO-, oxymyoglobin, and site directed mutagenesis and extracted two types of representative distortions to study in detail: the rotations of a pyrrole ring in the heme group and the imidazole in the His93 side chain.

II. COMPUTATIONAL METHOD

In the present study, we employ the *ab initio* pseudopotential method [12] with the local density approximation (LDA) [13] for the exchange-correlation effect. The Troullier-Martins-type pseudopotential [14] is adopted with the full nonlocality [15]. A standard plane wave code is used in the calculation. Because of the deep pseudopotential for the *d* orbital of the iron atom, a large energy cutoff of 70 Ry is used. Increasing the energy cutoff from 70 to 90 Ry changes the Hellmann-Feynman forces for each atom by less than 0.01 Ry/Å, which is within the tolerance of the present calculation.

It is not computationally feasible to include all the atoms in myoglobin or hemoglobin, and we cut out most of the protein environment and retain only the minimal cluster containing the heme group and the fifth ligand, as shown in Fig. 2. The outermost dangling bonds are passivated with hydrogen atoms. The model system is repeated in a simple cubic lattice of 13 Å. The nearest atom distance between the neighboring model systems (i.e., between one H atom in a model system and another in a neighboring model system) is 3 Å, which is sufficient for avoiding any direct interactions.

Structural relaxation is performed with the conjugate-gradient method [16] assuming a quadratic Born-Oppenheimer energy surface. In relaxing the free heme model we fix the position of the iron atom since the movement of the iron atom involves a large density fluctuation which makes the convergence very difficult. The usual partial core correction [17] is made to account for the nonlinear overlap effect between the frozen core and valence electrons. While the LDA is used for the electronic and ionic relaxation, a recently developed generalized gradient approximation (GGA) [18], as well as the LDA, are employed in evaluating the total energy to check the accuracy of the LDA with respect to the GGA.

III. RESULTS

A. The equilibrium geometry

First, we consider the equilibrium geometry of the model system in Fig. 2. The structural parameters of the relaxed geometry are shown in Table I. The calculated values are in good agreement with those from other theoretical [19] and experimental [20] results. We have also performed the calculation with the local spin density functional in order to find the spin state of the iron atom. The result is a closed shell singlet as in other theoretical works [19,21].

TABLE I. Structural relaxation of the free heme. All distances are in angstroms. See Fig. 2 for the definition of angles α and β. ΔE is the energy of the distortion with the LDA(GGA) functional. Distortions I and II mean the rotation of the pyrrole ring and the imidazole ring, respectively.

	Free model	Distortion I	Distortion II
d(Fe-N_p)	1.99	1.99	1.99
d(Fe-N_{Im})	1.96	1.97	1.99
d(C-O)	1.16	1.16	1.16
d(Fe-C)	1.79	1.79	1.78
α (°)	0	0	4.8
β (°)	0	3	3.5
ΔE(kcal/mol)	0	5.4(5.1)	7.4(7.2)

The calculated density of states (DOS) around the Fermi level is shown in Fig. 3. The energy gap between the LUMO and the HOMO state is 1.3 eV. The HOMO state is derived mainly from the iron d_{xy} orbital in the plane of four N atoms of the porphyrin cycle. Just below this state, there are iron d_{xz} and d_{yz} states π-bonded with p electrons of N atoms in the pyrrole ring and the imidazole as well as the CO molecule. The d_{xz} and d_{yz} orbitals are coupled with the p_z electrons of the pyrrole ring and lower in energy than d_{xy} by 0.5 eV because of the π bond. The other d orbitals, $d_{x^2-y^2}$ and $d_{3z^2-r^2}$, are higher in energy than d_{xy} by 2.7 and 2.9 eV, respectively. This is due to the electrostatic repulsion from the filled σ orbitals in the surrounding N atoms and the CO molecule. The LUMO state has the character of mainly π^*-bonded p_z orbitals in porphyrin macrocycle, weakly

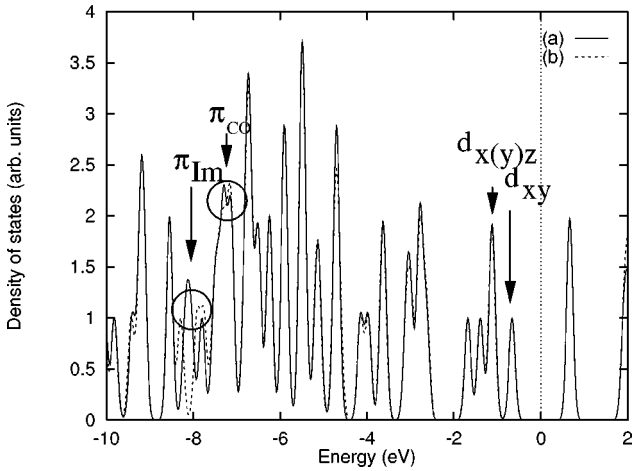

FIG. 3. Electronic density of states for the studied model systems with Gaussian broadening of 0.1 eV. Two curves correspond to the (a) free heme (with CO attached), and the (b) rotated-imidazole models, respectively. The DOS for the rotated-pyrrole model has been calculated as well, but is not presented because it is indistinguishable from that of the free model. The Fermi level is set to 0 eV. The circles indicate the region where the change in the DOS is significant.

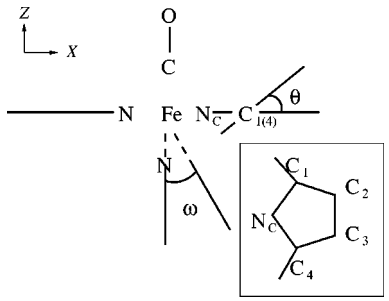

FIG. 4. Proximal distortions considered in this work. Inset is the top view of the rotated pyrrole C ring.

coupled with the iron $d_{x(y)z}$ orbital.

B. Rotation of a pyrrole ring

As noted above, the analysis on various PDB parameters reveals that the pyrrole C ring is rotated from the mean plane of the porphyrin around the C_1-C_4 axis in Fig. 4. The angle θ ranges from 5° to 8°. (see Table II). In practical situations, an ethene molecule is bounded to one end of the pyrrole C and directed inside the myoglobin and the rotation of the pyrrole C ring would be caused by the steric interaction of the ethene with other side chains. Since a flat geometry is more stable for a free iron porphyrin, the distortion of the heme cycle in myoglobin reflects the structural compactness of myoglobin. We simulate this distortion by rotating the pyrrole ring around the C_1-C_4 axis by 10° with ten outermost H atoms in the model heme group and imidazole fixed in the elevated position. Pinning of H atoms imitates the constraint imposed by the protein matrix.

The energy cost for this deformation is about 5 kcal/mol with both the LDA and GGA energy functional. The rotation of the pyrrole ring does not induce any significant structural changes in other parts. The N_c atom (Fig. 4) moves slightly downward (by 0.03 Å) because of the planar character of π-bonded states in the pyrrole, but it is far less than the expected value of $\sim$ 0.1 Å estimated from the x-ray data. The configuration of CO is almost unchanged from the linear geometry with small values of α and β, as shown in Table I. The nonzero β angle originates from a slight translation of oxygen to the positive y direction by 0.08 Å. An equivalent motion of CO into the negative y direction would also be possible since the deformation conserves the mirror symmetry with respect to the xz plane. The DOS does not show any appreciable changes from the unperturbed iron porphyrin model and is omitted in Fig. 3. We only find a tiny shift to a higher energy around -7 eV, which is related to the bonding disruption of the pyrrole ring caused by the rotation.

TABLE II. Rotation angles of the pyrrole and the imidazole ring (defined in Fig. 4) for various PDB parameters. Owing to a low resolution of the x-ray crystallography for protein, measured rotation angles can vary rather widely.

PDB	2MB5	1MBC	1ABS	1MLF	1MLQ	1MOC
θ (°)	8.0	6.8	7.3	5.0	7.3	5.2
ω (°)	6.7	1.1	2.5	−0.2	0.4	0.5

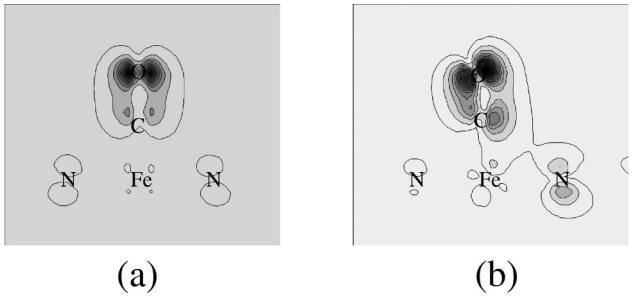

FIG. 5. Charge density contour plot of the state involved in the DOS change around -7.5 eV in Fig. 3, for the (a) free model, and for the (b) rotated-imidazole model. The successive contours are separated by $0.01e$/a.u.[3]

C. Rotation of the imidazole side chain

In the theoretical works by Jewsbury *et al.* [10] and Ghosh and Bocian [11], the rotation of the imidazole chain around the x axis (Fig. 4) was studied. In this study, we consider the rotation around the y axis which is significant in some proteins as shown in Table II. The rotation of the imidazole ring is initiated with the translation of the bottom H atoms in the ring by 0.5 Å in the x direction and the whole geometry is relaxed except for the outermost H atoms for the same reason as mentioned in the preceding section.

Upon relaxation, the imidazole ring is rotated so that the nitrogen atom is directed toward the iron atom and the rotation angle ω reaches 5.8°. The position of the iron atom is almost unchanged. The energy cost is 7.4(7.2) kcal/mol with the LDA(GGA) energy functional. Unlike the previous case, the geometry of the CO molecule after relaxation now changes rather significantly so that α and β acquire relatively large values, 4.8° and 3.5°, respectively.

In order to understand the microscopic origin of the observed bending and tilting of the CO molecule, we have analyzed the change in the electronic structure around the iron atom. In Fig. 3, we find that the DOS inside the circles is significantly different from the other two previous systems. The change around -8 eV is related to the orbitals at the N atoms of the imidazole and does not contribute to the CO distortion. An apparently small change in the DOS at -7.5 eV actually involves a drastic change in the character of p_x electrons in the CO molecule as demonstrated in Figs. 5(a) and 5(b). Because of the $dp\pi$ bonding between the imidazole and the iron atom, the rotation of the imidazole induces the rotation of the d_{xz} orbital and generates a partially $d_{3z^2-r^2}$ character. The carbon atom slightly moves to the $-x$ direction following the rotation of the d_{xz} orbital. However, since the other orbital (d_{yz}) bonded to carbon (p_y) is not changed, the carbon atom cannot fully follow the rotation and the symmetry of the charge density of the carbon p orbital with respect to the C-Fe axis is broken, i.e., the charge is more accumulated on the right-hand side of the C atom in Fig. 5(b). In order to compensate for the charge depletion on the left-hand side of the C atom arising from this broken symmetry, the p_x orbital of the O atom slightly rotates counterclockwise in the figure and the nonzero β angle is generated from this process.

We could not find the expected correlation between the CO bending angle and the distortion of the imidazole directly in PDB parameters. As noted in earlier theoretical works

[10], this might be attributed to a low resolution of the x-ray image. Comparing two types of distortions studied above, we find that the movement of N atoms surrounding the iron atom is important for the rotation of the iron *d* orbital. In the rotation of the pyrrole ring, the N atom movement is negligibly small (0.03 Å) and the configuration of CO is unchanged, whereas the rotation of the imidazole ring involves the N atom displacement of 0.2 Å and CO is significantly bent. The calculated bending energies of the CO molecule in the model free heme are 1.9, 5.7, and 8.0 kcal/mol for 10°, 20°, and 30°, respectively. Since the $\alpha+\beta$ angle turns out to be 8.3° in this work, the affinity reduction is estimated from the extrapolation to be around 1.5 kcal/mol. This value, however, is an order of magnitude too small to explain the whole reduction ($\sim 10^3$) in relative affinity of CO compared to O_2, and the rotation of the imidazole ring alone cannot explain the experimental affinity reduction.

IV. SUMMARY

In summary, we have investigated the effect of proximal distortions around the iron atom on the CO geometry using the *ab initio* LDA method including GGA as a perturbation. We have found that the orientational distortion of the imidazole simulating the His93 in myoglobin has a significant effect on the d_{xz} orbital of the iron atom, thereby changing the geometry of CO attached to it. In contrast, the rotation of the pyrrole C ring does not significantly influence the geometry of the CO molecule.

Regarding the issue of the CO bending and its affinity to the iron porphyrin, we could not observe a large bending angle reported in some x-ray crystallography studies. However, we cannot rule out the possibility that a larger distortion or some combination of different types of distortions might lead to the bending angle as large as that observed in some experiments. Alternatively, since the surface area of the molecule is quite large, small strains induced by the crystal packing force can accumulate and cause large distortions observed in PDB parameters. In solvent situation where an external constraining force is absent, the imidazole attached to a heme group will fluctuate around the equilibrium position. Since the direction of the CO bending is expected to follow this fluctuation according to our calculation, the bent geometry might be unobservable in the physiological condition as in a recent IR experiment [7].

From the energetic point of view, the distortion studied in this paper is easily realizable in experimental situations, and our findings can be applied to a design of artificial chemical sensors using metalloporphyrins. If the sensors are arrayed in crystalline phase, it will be possible to apply external stress or strain to force some distortions in the heme group. The shape of an individual porphyrin molecule can be changed intentionally by appropriate interactions with the substrates.

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