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Toward Modeling H-NOX Domains: A DFT Study of Heme-NO Complexes as Hydrogen Bond Acceptors

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Density functional theory calculations (PW91/STO-TZP, including basis-set superposition error corrections) have been used to evaluate hydrogen bond energies of five- and six-coordinate heme-NO complexes with phenol and imidazole, chosen as models for distal pocket tyrosine and histidine residues. The calculated interaction energies are approximately 2 kcal/mol for phenol and 3–4 kcal/mol for imidazole, which are 2–4 times smaller than the energies calculated for heme-O_2 complexes hydrogen-bonding with a distal histidine. Interestingly, the hydrogen bond energies are found to be very similar for five- and six-coordinate heme-NO complexes, which may be viewed as contrary to the interpretation of a recent observation on a bacterial H-NOX (*H*eme-*N*itric oxide/*OX*ygen-binding) protein with sequence homology to mammalian-soluble guanylate cyclase.

Introduction

The mammalian NO-sensing enzyme soluble guanylate cyclase (sGC) catalyzes the conversion of GTP to cyclic GMP (cGMP) and thereby plays an important role in cGMP-mediated signaling pathways.[1,2] Thus, NO and sGC play a key role in a variety of physiological processes, notably vasodilations. A most intriguing aspect of sGC as an Fe(II) heme protein is that it selectively binds NO (and CO) but not O_2. Compared with the respiratory proteins myoglobin and hemoglobin, how does sGC achieve this "reverse" discrimation among the diatomic ligands?[3] The recent discovery and structural characterization[4−7] of a number of bacterial proteins (named H-NOX for *H*eme-*N*itric oxide/*OX*ygen-binding)[3,4] with sequence homology to sGC have shed significant light on this question. Like sGC (which has not yet been structurally characterized), *certain* of these bacterial orthologs also exclude O_2 under aerobic conditions while binding NO selectively; unlike the respiratory heme proteins, these do not have a hydrogen bond donor in the distal pocket, which could potentially stabilize a heme-bound O_2.[3] However, certain H-NOX proteins found, curiously enough, in obligate anerobic bacteria *do* have a strategically positioned distal pocket tyrosine (or other hydrogen bond donor), and these reversibly bind O_2 as well NO and CO. Marletta and co-workers have suggested that the O_2 binding regulates downstream chemical events, which, in turn, lead to taxis toward regions of lower O_2 concentration. Here we have made a preliminary attempt to model the interaction of a distal pocket hydrogen bond donor with heme-bound NO.

Raman and co-workers have reported an interesting observation on the coordination behavior of a recombinant version (named CB-$SONO_{HD}$) of an H-NOX protein from *Clostridium botulinum*, which has a distal pocket tyrosine (Y139).[6] According to electronic paramagnetic resonance (EPR) spectroscopic evidence, CB-$SONO_{HD}$ binds NO to yield a five-coordinate (5c) heme-NO complex, where the proximal ligand has fallen off.[6] However, the distal pocket Tyr → Phe mutant binds NO to yield a six-coordinate (6c) heme-NO complex.[6] The potential implication of this remarkable observation is perhaps best conveyed in the authors' own words:[6] "The ability of an amino acid side chain in the distal pocket to modulate the bond strength of the proximal Fe−His linkage is unparalleled in heme protein research. ... these results ... suggest that electrostatic interaction with Tyr139 is necessary for generating neet [sic] 5c-Fe(II)NO complex and subsequent breaking of the Fe−His bond." Naturally, the question arises as to the generality of

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the phenomenon. Why do we have a 5c complex in the presence of a distal tyrosine? Does distal hydrogen bonding necessarily weaken the proximal Fe−His linkage? Is a 5c heme-NO complex an inherently better hydrogen bond acceptor than a 6c complex?

Recently, Richter-Addo and co-workers have reported a 1.9-Å horseheart myoglobin-NO crystal structure[8] that apparently confounds the above argument: here a *6c* heme-NO complex clearly forms a hydrogen bond with a distal histidine residue (His64). Interestingly, the His64 NH interacts with both the nitrogen and oxygen atoms of the NO ligand, with the interaction with the NO nitrogen being closer. In contrast, for the H-NOX-O_2 structures reported to date, the only the terminal oxygen of the heme-bound O_2 interacts with a distal pocket tyrosine.[5−7]

In light of the above observations and speculations, we undertook a quantum chemical study with a view to answering the following questions: Does a coordinated NO favor a particular geometrical arrangement of hydrogen bonds? More specifically, does the coordinated NO prefer to hydrogen-bond via the N or the O?[8] How much energy is associated with these hydrogen bonds? Is there a significant difference between 5c and 6c heme-NO complexes as hydrogen bond acceptors? In this study, we have attempted to answer these questions via density functional theory (DFT) calculations on relatively simple heme active site models.

Computational Methods. All calculations were carried out with the PW91 functional for both exchange and correlation, STO-TZP basis sets, tight criteria for self-consistent-field convergence and geometry optimization, and a very fine integration mesh, as implemented in the ADF 2004 program system.[9] 5c and 6c heme-NO active sites were modeled with Fe(P)(NO) and Fe(P)(NO)(ImH) (P = porphine, ImH = imidazole), respectively.[10] For the H-NOX models, a distal pocket tyrosine was modeled with phenol (PhOH) and only hydrogen bonding via the NO oxygen was considered. A distal pocket histidine was modeled by an ImH, and two different hydrogen bonding geometries were considered for both of our 5c and 6c heme-NO models.

The energies associated with hydrogen bonding were corrected for basis-set superposition error (BSSE) by the counterpoise method. We also accounted for the fact that the isolated hydrogen bond donor and acceptor molecules undergo small geometrical changes as they form a hydrogen-bonded supermolecule. In general, the BSSEs were less than 5−10% of the hydrogen bond interaction energies. For selected systems, improving the basis set to TZDP and QZDP+d resulted in minimal changes (<0.2 kcal/mol) in the hydrogen bond energies, implying that the TZP energies themselves are relatively well-converged with respect to improving the basis set.

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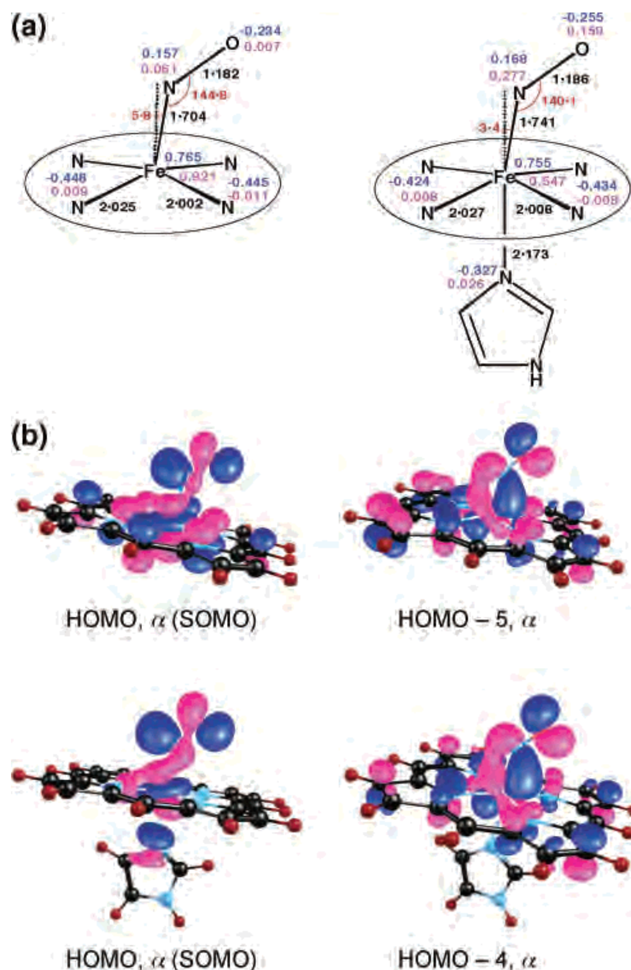

Figure 1. Selected calculated results on Fe(P)(NO) and Fe(P)(ImH)(NO). (a) Selected distances (Å, in black), angles (deg, red), Mulliken charges (blue), and spin populations (magenta), including a definition of the tilt and bend angles. (b) Selected a′ molecular orbitals.

Results and Discussion

(a) Some Fundamentals of Heme-NO Bonding. Before we present our results that explicitly address the questions posed above, it is useful to review some of the fundamentals of a heme-NO electronic structure. Figure 1 presents some key calculated results on Fe(P)(NO) and Fe(P)(ImH)(NO). The optimized geometrical parameters are in generally good agreement with experimental metrical parameters on related 5c and 6c iron porphyrins and do not merit extensive comment.[11−13] However, we will briefly mention three points. (a) Note that the presence of a sixth ligand (ImH) pushes much of the spin density away from the Fe onto the NO. This has long been appreciated from EPR studies.[14] (b) Second, the $Fe-N_{ImH}$ bond is rather long, a reflection of NO's trans effect or, more specifically, of the antibonding metal (d_{z^2})−ImH interaction present in the highest occupied

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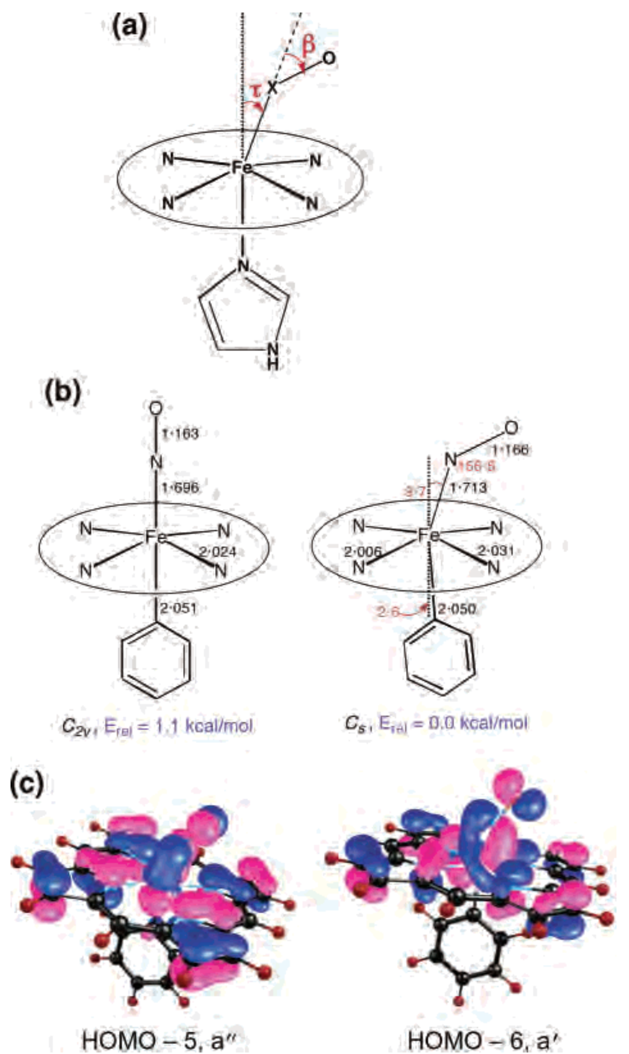

Figure 2. (a) Definition of the tilt (τ) and bend (β) angles. Highlights of calculated results on Fe(P)(Ph)(NO): (b) selected distances (Å, in black) and angles (deg, red); (c) selected molecular orbitals for the C_s geometry.

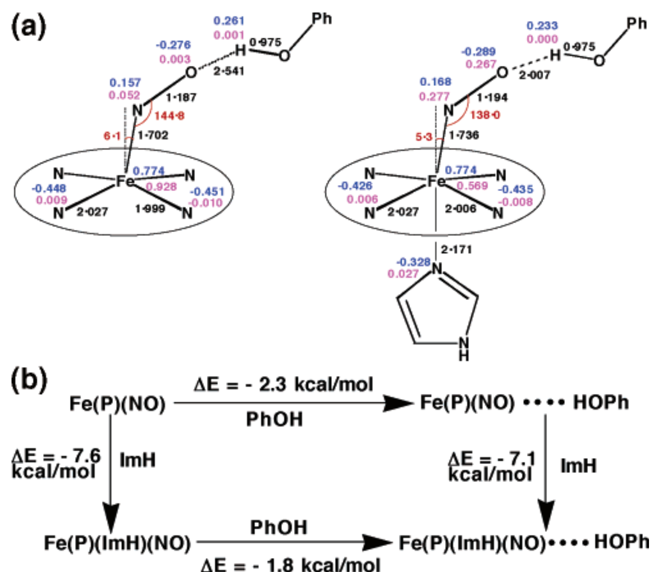

Figure 3. Highlights of calculated results on Fe(P)(NO)···HOPh and Fe(P)(NO)(ImH)···HOPh. (a) Selected distances (Å, in black), angles (deg, red), Mulliken charges (blue), and spin populations (magenta). (b) Calculated energetics.

molecular orbital (HOMO), which is the singly occupied molecular orbital, of Fe(P)(ImH)(NO) (Figure 1). (c) Third, the Fe–N_{NO} vector is tilted relative to the heme normal, which has also been observed experimentally;[11–13] this may seem paradoxical, given that the Fe d_{z^2} orbital component of the HOMO (Figure 1) tilts in the *opposite* direction relative to the heme normal. Why then does the Fe–N_{NO} vector tilt the way it does? The answer lies in another lower-energy orbital interaction, namely, the a′Fe(d_π)–NO(π*) π interaction (also shown in Figure 1), which apparently wins out.

Digressing slightly, a similarly curved a′Fe(d_π)–NO(π*) orbital interaction is also seen for Fe(P)(Ph)(NO), as shown in Figure 2. Like the heme-NO structures described above, the optimized structure of this molecule also exhibits cooperative tilting and bending (see Figure 2a for a definition of these angles), which is unique for $\{MXO\}^6$ porphyrins but, fortunately, also in excellent agreement with experimental results on M(P)(Ar)(NO) (M = Fe, Ru).[15] From Figure 2, note that the C_{2v} symmetry-constrained conformation of Fe(P)(Ph)(NO) is only about 1 kcal/mol higher in energy than the C_s conformation. This should not be surprising given that cooperative tilting and bending has long been known to provide a low-energy deformation pathway (as well as a low-frequency vibrational mode) for $\{MXO\}^6$ porphyrins, where once again the same orbital interaction accounts for the observed flexibility.[16–19]

(b) Modeling H-NOX Active Sites. Figure 3 presents the optimized geometries for Fe(P)(NO)···HOPh and Fe(P)(NO)-(ImH)···HOPh, where we have chosen PhOH as a model for a distal tyrosine side chain, as well as the calculated energetics results, which have been corrected for BSSE by the counterpoise method. In general, the BSSEs were less than 10% of the hydrogen bond interaction energies. As shown in Figure 3a, hydrogen bonding is present for both the 5c and 6c heme-NO models, resulting in a small increase in the NO distances (relative to Figure 1).

Our key result is that *both 5c and 6c heme-NO complexes may act as hydrogen bond acceptors*; *with PhOH as the hydrogen bond donor, the hydrogen bond energy is found to be 1.8–2.3 kcal/mol in either case*. These energy ranges remained unchanged for TZP, TZDP, and QZDP+d STO basis sets. As expected, this energy is about 3–4 times lower than that computed for hydrogen bonding between an oxyheme model and a distal ImH.[20] The results shown in Figure 3 also suggest that *distal hydrogen bonding has little effect on the energetics of the proximal Fe–His linkage*. On the basis of these results, the observation by Raman and co-

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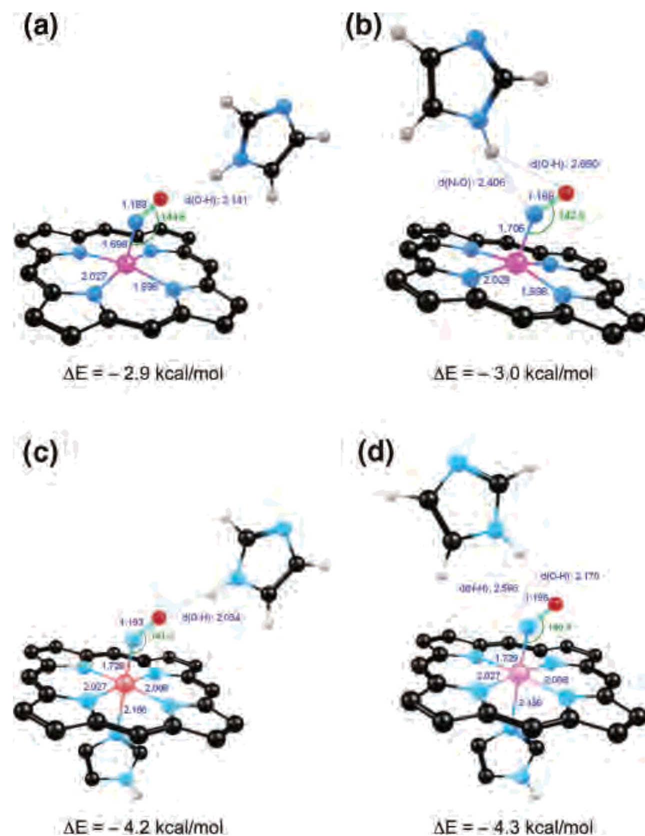

Figure 4. Optimized geometries of 5c and 6c heme-NO complexes hydrogen bonded to an ImH. Porphyrin and proximal hydrogens are not shown. Also shown are the TZP BSSE-corrected hydrogen bond energies.

workers (quoted above),[6] while interesting, does not appear to be general but seems specific for the particular protein they studied. Phrased differently, our calculations do allow the possibility of distal pocket hydrogen bonding involving 6c heme-NO cofactors, something that we look forward to seeing as additional H-NOX crystal structures are reported.

(c) Alternative Hydrogen Bonding Geometries. To explore the question of whether a heme-bound NO prefers to hydrogen-bond via the NO nitrogen or oxygen,[8] we carried out four different geometry optimizations involving a "distal" ImH, the results of which are shown in Figure 4. Figure 4 also shows the BSSE-corrected interaction energies involved. The following are some of the more notable conclusions that we may draw.

First, note that the hydrogen bond energies involving ImH are about 1.5−2 times those involving PhOH as the hydrogen bond donor.

Second, the hydrogen bond energies obtained with the 6c heme-NO model are about 1 kcal/mol greater than those obtained with the 5c model.

Third, for both 5c and 6c heme-NO complexes, the two alternative hydrogen bonding geometries examined are nearly identically favored in energy terms. In other words, depending on whether a distal pocket hydrogen bond donor is on the same side of the heme normal as the bent NO or not, the hydrogen bond formed will involve only the NO oxygen or both the NO nitrogen and oxygen, respectively.

Conclusion

In conclusion, DFT calculations indicate that both 5c and 6c heme-NO are comparable hydrogen bond acceptors. For a distal tyrosine (PhOH) as the hydrogen bond donor, the BSSE-corrected interaction energies involved are about 2 kcal/mol or perhaps somewhat less (assuming the DFT calculations have slightly overestimated true interaction energies[20]). For a distal pocket histidine (ImH), the calculated hydrogen bond energies are about 3−4 kcal/mol, which is about half the interaction energy calculated for a heme-O_2 complex and a distal histidine (ImH). Thus, in the case of some of the H-NOX proteins studied, it is possible that a distal tyrosine (as opposed to a histidine) residue provides just the right balance of NO versus O_2 affinity that is consistent with a possible chemotactic[3] function of these proteins. Overall, our results support the emerging view that protein electrostatics and hydrogen bonding interactions, as opposed to steric effects, are the key factors controlling diatomic ligand discrimination by heme proteins, including sGC and the other H-NOX proteins.[3,21]

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Supporting Information Available: Table of optimized Cartesian coordinates. This material is available free of charge via the Internet at http://pubs.acs.org.

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