

Cytochrome P450: An Investigation of the Mössbauer Spectra of a Reaction Intermediate and an Fe(IV)=O Model System

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High-valent iron-oxo species are important intermediates in the catalytic cycles of many heme enzymes, and there have been numerous reports describing their geometric and electronic structures, spectroscopic properties, and associated biochemical reactions.^{1–11} Many of these studies have focused on Fe^{IV}=O systems containing either histidine or cysteine as the sixth ligand, as found in peroxidases^{3–5} and cytochromes P450,^{6–8} but there is still no consensus picture of their structures. For example, the Fe=O bond lengths found vary from 1.64 to 1.75 Å,^{3–8} while the (optimized) Fe–S bond lengths in a P450 Fe=O reaction intermediate (P450-RI) vary from 2.37 to 2.71 Å.^{6–8} There is thus considerable interest in investigating, both experimentally and theoretically, the structures of much smaller model systems, and recently the crystallographic structure of a non-heme Fe(IV)=O complex was reported.¹ Its Fe–O bond length is 1.646 Å, a little shorter than that found in a putative P450-RI protein structure (1.669 Å).² There is also debate about the spin state for the P450-RI: does it consist of antiferromagnetically (or ferromagnetically) coupled Fe(IV)=O ($S = 1$) and porphyrin ($S = 1/2$) radicals,^{6–8} or is an amino acid residue of the protein involved?¹⁰ Questions as to the protonation state of the axial cysteine and dynamic proton transfer have also been raised.⁹

Based on our work with Fe–CO and Fe–NO bonding in heme proteins,^{12a,b} it seemed possible that the origins of some of these uncertainties might be related to the difficulties in obtaining accurate bond length and bond angle results in large proteins. We previously found that while the Mössbauer spectra (⁵⁷Fe quadrupole splittings, ΔE_Q , and isomer shifts, δ_{Fe}) of small model compounds could be well predicted by using density functional theory (DFT),¹³ the spectra of some proteins could not be accurately predicted, unless geometry optimization techniques were used.¹² We have therefore now applied the DFT approach to investigate the ⁵⁷Fe Mössbauer spectra of both a model Fe^{IV}=O system, [Fe(O)(TMC)(NCCH₃)]-(OTf)₂ (TMC = 1,4,8,11-tetramethyl-1,4,8,11-tetraazacyclotetradecane, OTf = CF₃SO₃[−]), as well as a P450-RI. Unlike our previous studies of Fe–CO and Fe–NO bonding in heme proteins, there are many more possibilities for spin state, oxidation state, protonation state and geometry in the P450-RI, but DFT methods enable the investigation of each effect on ΔE_Q and δ_{Fe} and, when compared with experiment, give important clues as to the nature of structure and bonding in this system.

We first investigated the Fe^{IV}=O model system, [Fe(O)(TMC)(NCCH₃)]²⁺, using a recently published high-resolution X-ray structure.¹ Calculations were carried out using basically the same basis set scheme as reported previously,^{13,14} and both BPW91 and B3LYP functionals were investigated.¹⁵ For the spin-state $S = 1$, the predicted ΔE_Q and δ_{Fe} values were 1.24 (1.25) and 0.20 (0.13) mm s^{−1} using the BPW91 (B3LYP) functional, basically the same as observed experimentally: 1.24 (ΔE_Q) and 0.17 mm s^{−1} (δ_{Fe}).¹

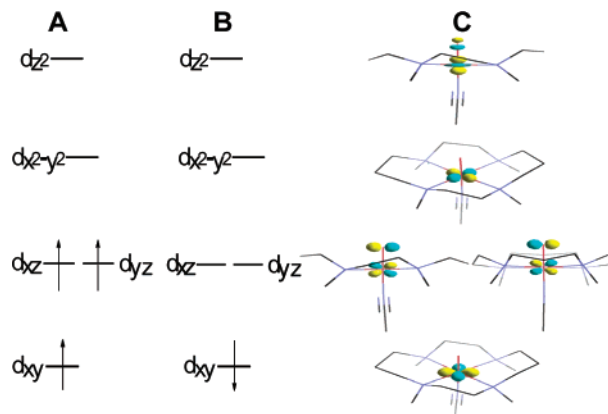


Figure 1. Frontier orbital occupancy from the crystal field analysis for α -spins (A) and β -spins (B) together with (C) frontier MOs from DFT calculations for both spins of [Fe(O)(TMC)(NCCH₃)]²⁺. The iso-surface values in (C) are ± 0.2 au.

Calculations using an alternative spin state ($S = 2$) resulted in a much smaller ΔE_Q : 0.42 (BPW91) or 0.53 (B3LYP) mm s^{−1}. The predicted isomer shifts were also inferior to the $S = 1$ results: 0.13 (BPW91) or 0.097 (B3LYP) mm s^{−1}. Moreover, the $S = 2$ state was less stable than the $S = 1$ state by 103.76 (BPW91) or 64.70 (B3LYP) kJ mol^{−1}. So, these DFT calculations strongly support a designation of $S = 1$ in this Fe^{IV}=O system, consistent with expectation.¹ Similar results were obtained using a geometry optimized structure (Table S1, Supporting Information). The frontier molecular orbitals (MOs) from a crystal field analysis are shown in Figure 1A and 1B (for the α and β orbitals, respectively), and the DFT MO results, Figure 1C, generally support this analysis. However, there is also a significant covalent contribution from the oxo group interacting with the iron 3d_{xz}, 3d_{yz}, and 3d_{z²} orbitals (Figure 1C). The calculated spin densities for iron and oxygen are 1.2 and 0.8 au, respectively, in good accord with previous DFT calculations on Fe^{IV}=O porphyrins.^{3,7–8} These results serve to validate the use of DFT methods in predicting Mössbauer ΔE_Q and δ_{Fe} results in this Fe^{IV}=O model system and supplement the ΔE_Q , δ_{Fe} results on other model systems reported previously.^{13,16} Next, we investigated the prediction of the ⁵⁷Fe Mössbauer ΔE_Q and δ_{Fe} results for the P450-RI. The experimental data^{10a} for the P450-RI detected during an 8-ms reaction time are $\Delta E_Q = 1.94$ mm s^{−1} and $\delta_{Fe} = 0.13$ mm s^{−1}, and the ESR results indicated an $S = 1$ species.¹⁰ In our calculations, however, we investigated a variety of spin states ($S = 1/2, 1, 3/2$) as well as iron and porphyrin oxidation states, together with the protonation states of the coordinated cysteine (Table 1, 1–7). For convenience, we use the XYZ designation: Fe^{X+}O^{2−}(Por)^{Y−}(Cys)^{Z−}, with $X = 4$ or 5, $Y = 1$ or 2, and $Z = 0$ or 1 (CH₃SH or CH₃S[−]). For clarity, we include only

Table 1. Computational Results for Fe^{X+}O²⁻(Por)^{Y-}(Cys)^{Z-} Complexes Using a BPW91 Functional

| system | (X,Y,Z) | R _{FeO} (Å) | ∠O–Fe–S (deg) | R _{FeS} (Å) | E ^d (kJ mol ⁻¹) | ρ _{orb} ^{Fe} (e) | ρ _{orb} ^O (e) | ρ _{orb} ^S (e) | ΔE _Q (mm s ⁻¹) | δ _{Fe} (mm s ⁻¹) |
|-------------------------------|---------|-------------------------|------------------|-------------------------|---|---------------------------------------|--------------------------------------|--------------------------------------|--|--|
| expt. ^a | | 1.669 | 165.8 | 2.271 | | | | | 1.94 | 0.13 |
| 1 S = 1/2 ^b | 4, 1, 1 | 1.655 | 167.4 | 2.364 | 1632.62 | 0.95 | 0.77 | -0.51 | -1.08 | 0.08 |
| c | | | | | (1724.68) | (0.77) | (0.85) | (-0.53) | (-1.37) | (0.08) |
| 2 S = 3/2 ^b | 4, 1, 1 | 1.673 | 172.4 | 2.477 | 1649.18 | 1.26 | 0.92 | 0.57 | 0.35 | 0.18 |
| c | | | | | (1748.17) | (1.36) | (1.02) | (0.55) | (-1.08) | (0.14) |
| 3 S = 1 ^b | 4, 2, 1 | 1.699 | 176.9 | 2.439 | 1395.31 | 1.16 | 0.87 | 0.05 | 0.12 | 0.26 |
| c | | | | | (1493.14) | (1.12) | (0.88) | (0.11) | (-0.67) | (0.23) |
| 4 S = 1 ^b | 5, 1, 1 | 1.648 | 172.3 | 2.356 | 2270.89 | 0.90 | 0.81 | -0.51 | -1.04 | 0.06 |
| c | | | | | (2364.84) | (0.77) | (0.91) | (-0.55) | (-1.25) | (0.09) |
| 5 S = 1/2 ^b | 4, 1, 0 | 1.632 | 171.5 | 2.602 | 627.93 | 1.05 | 0.75 | -0.09 | 1.79 | 0.08 |
| c | | | | | (756.20) | (1.00) | (0.88) | (-0.05) | (0.72) | (0.15) |
| 6 S = 3/2 ^b | 4, 1, 0 | 1.654 | 175.1 | 2.621 | 626.72 | 1.39 | 0.91 | 0.03 | 0.59 | 0.09 |
| c | | | | | (764.55) | (1.23) | (1.04) | (0.01) | (-0.60) | (0.16) |
| 7 S = 1 ^b | 4, 2, 0 | 1.650 | 176.0 | 2.708 | 0.00 | 1.19 | 0.86 | -0.02 | 1.69 | 0.17 |
| c | | | | | (136.28) | (1.08) | (0.99) | (-0.01) | (0.64) | (0.19) |

^a The experimental X-ray structure and Mössbauer data are from refs 2 and 10a, respectively. ^b Fully optimized geometry (BPW91). ^c Experimental geometry (PDB file 1DZ9). ^d Energies are referenced to the most stable species (**7**, -2766.32510 au) for convenience.

the BPW91 calculated properties in Table 1, but B3LYP results (Table S2) are very similar. When using the coordinates of a P450-RI structure,² none of the DFT calculations yielded accurate predictions for ΔE_Q, as shown by the data in parentheses in Table 1. We therefore next used a series of geometry optimized structures (**1–7**, Supporting Information), since, in previous work with Fe–CO and Fe–NO systems, we have found that this approach enables the accurate prediction of Mössbauer, as well as NMR and EPR observables.¹² As shown in Table 1, none of the ΔE_Q predictions for the thiolate (Z = 1) species (**1–4**) are consistent with the experimental ΔE_Q value. The closest result is 0.35, to be compared with the experimental result of ΔE_Q = 1.94 mm s⁻¹.^{10a} Since these four species encompass all of the commonly proposed spin/oxidation states (including porphyrin radicals), we next considered the possibility of a protonated cysteine,⁹ with all three spin states: S = 1/2, 3/2, for the porphyrin radical cations, **5,6**; S = 1 for the Fe^{IV}–porphyrin dianion, **7**; Table 1. The ΔE_Q results are now all much larger, and, for **7**, the lowest energy thiol species which also has S = 1, as indicated experimentally,¹⁰ the predicted results using both BPW91 (Table 1) and B3LYP (Table S2) are, within the expected uncertainties¹³ (ΔE_Q error ~0.3 mm s⁻¹, δ_{Fe} error ~0.1 mm s⁻¹), the same as those found experimentally. For **7**, the computed R_{FeO} of 1.650 Å is essentially that found in the model complex (1.646 Å), as are the O–Fe–X bond angles (176.0°, ∠O–Fe–S, **7**; 178.9°, ∠O–Fe–N, model complex¹). The R_{FeS} value is, of course, considerably larger than that reported crystallographically; however, the short value reported may be due, at least in part, to refinement using a thiolate ligand,² and indeed our geometry optimization result on **3** (R_{FeS} ≈ 2.4 Å) is close to that reported² (R_{FeS} ≈ 2.3 Å, Table 1). Neutral sixth ligands are favored in Fe=O model complexes and peroxidases with positive ΔE_Q,¹¹ and a protonated (or strongly hydrogen bonded) cysteine may help explain the redox potential in P450.¹⁷ Moreover, our results do not rule out the possibility of an additional thiolate reaction intermediate (e.g., **3**), arising from a dynamic proton transfer,^{9b} but this species does not appear to give rise to the experimental Mössbauer results observed by Schünemann et al.^{10a,b}

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Supporting Information Available: Computational results for optimized [Fe(O)(TMC)(NCCH₃)₂]²⁺ (S = 1), B3LYP-calculated properties for different P450-RI models, and the geometries of all optimized structures (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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