

## Medium Polarization and Hydrogen Bonding Effects on Compound I of Cytochrome P450: What Kind of a Radical Is It Really?

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Compound I of Cytochrome P450 (the iron–oxo species in Scheme 1) is the primary oxidant that enables the enzyme to perform its vital roles in biosystems. The species of P450<sub>cam</sub> was tentatively assigned by means of trapping X-ray cryo-crystallography,<sup>1</sup> but most of its features are still largely uncharacterized. This paucity concerns mostly the Fe–S bond, between iron and the proximal ligand, which is estimated to be responsible for the stability of compound I against reduction and for its reactivity in mono-oxygenation reactions.<sup>2</sup>

Theoretical calculations of compound I models have so far been limited to the bare system without taking into account the polarization effects and specific interactions exerted by the amino acid groups in the protein pocket.<sup>3</sup> Indeed, Poulos et al.<sup>4</sup> have demonstrated that the thiolate ligand is coordinated in the proximal pocket by two NH–S<sup>−</sup> hydrogen bonds, and is stabilized by exposure to the side of the helix that contains the positive ends of the polar groups. The studies of Dawson et al.<sup>5</sup> further demonstrate the importance of these interactions to stabilize the Fe–S linkage which otherwise does not form. Model systems by Suzuki et al.<sup>6</sup> showed that NH–S<sup>−</sup> hydrogen bonds affect the mono-oxygenase activity of the model complex. Hydrogen bonds in the distal pocket seem to play a role by stabilizing the FeO moiety of compound I of chloro-peroxidase (CPO).<sup>7</sup> Such an FeO–HOH bond may attend the formation of compound I, either with the specific water molecule that is eliminated from the hydroperoxo complex<sup>2b,d</sup> to generate compound I or with the disordered water population in the distal pocket.<sup>1</sup> We therefore deemed it necessary to study the effect of hydrogen bonding and medium polarization on electronic and geometric features of compound I, with an attempt to resolve disparities and enhance the useful interplay of theory and experiment. *As will be seen, the hydrogen bonding and medium polarization exert a dramatic effect on the electronic structure and stabilize the Fe–S bond.*

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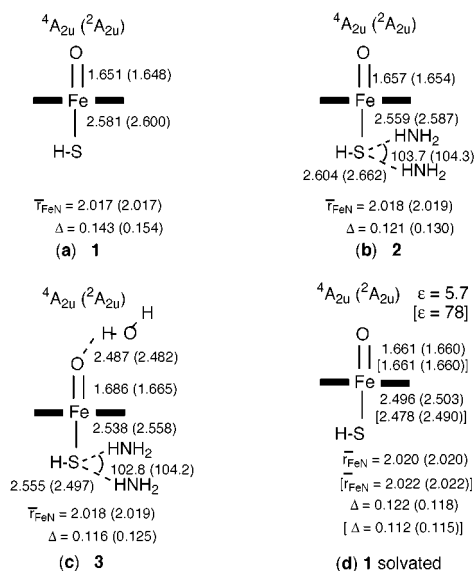
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## Scheme 1. Key Parameters (Å and deg) of Compound I (iron-oxo)



Modeling of the environmental effects was carried out by a comparison of the properties of the bare compound I species **1** with hydrogen-bonded species, **2** and **3** (Scheme 1). All the species have been studied as such, as well as under the influence of solvent polarization, using solvent models with a range of dielectric constants of  $\epsilon = 5.7$  and 78 designed to probe the effect over a range of reaction field and medium polarization strengths. In all the species we used HS<sup>−</sup> as the proximal ligand since it was proven to be a better mimic of the full cysteinato ligand compared with CH<sub>3</sub>S<sup>−</sup>, reproducing the state spacing and Fe–S bond energy of the full cysteinato ligand.<sup>3d,e</sup> Nevertheless, it must be stressed that **1** is a truncated model system. Calculations were done with the GAUSSIAN98 package,<sup>8</sup> using the unrestricted hybrid functional, UB3LYP,<sup>9</sup> with full geometry optimization and frequency analysis. An effective core potential and the double- $\zeta$  quality LACVP basis set<sup>10</sup> were used for iron and 6-31G for all other atoms, hence LACVP-6-31G. This basis set was proven qualitatively reliable in our recent extensive study of a few basis sets.<sup>3d,11</sup> Solvent calculations were carried out with the PCM model implemented in JAGUAR 4.0.<sup>12</sup> Only the two lowest spin states were computed, and are assigned hereafter as <sup>2,4</sup>A<sub>2u</sub>, following previous nomenclature.<sup>3d,e</sup>

Scheme 1 shows the key geometric parameters for the lowest states of compound I under different conditions.<sup>11</sup> Part (a) shows the parameters in the bare system, while parts (b) and (c) show the situations with hydrogen bonding to the thiolate and the oxo-group, respectively. The main effect is a shortening of the Fe–S bond, a somewhat less significant elongation of the FeO bond, and a gradual landing of the iron into the porphyrin plane given by the distance  $\Delta$ . A much more significant change is exerted by the solvent polarization shown in part (d) of Scheme 1. Thus, for both values of the dielectric constant, the Fe–S bond is shortened

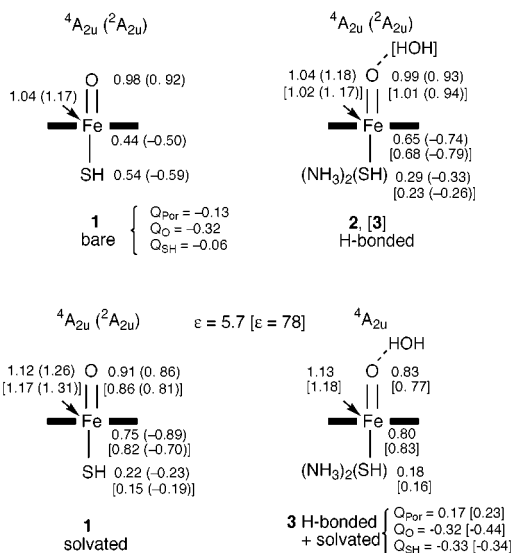
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(11) Results with extended basis sets are virtually the same. The Fe–S, Fe–O, Fe–N, and  $\Delta$  structural parameters (in Å) of, e.g., the <sup>4</sup>A<sub>2u</sub> state of I in a vacuum (in C<sub>6</sub>Cl<sub>6</sub>) are the following respectively for the polarized double- $\zeta$  LACVP\*-6-31G\* and polarized triple- $\zeta$  LACV3P\*-6-311G\* basis set: 2.579 (2.487), 1.626 (1.636), 2.019 (2.022), 0.167(0.147) and 2.567 (2.473), 1.631 (1.642), 2.018 (2.021), 0.139 (0.112). LACV3P/6-311G and LACVP-6-31G gave identical parameters.

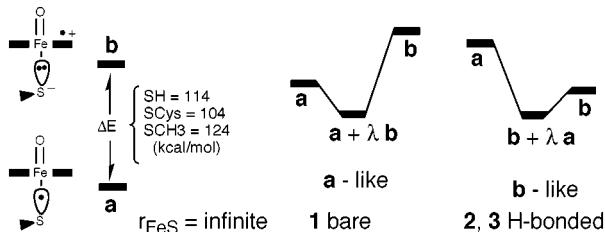
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**Scheme 2.** Group Spin Densities and Charges

by ca. 0.1 Å, while the other parameters are less affected. The significant tightening of the FeS bond has been predicted by us recently, showing that shortening of the bond between 2.6 and 2.44 Å costs less than 1 kcal/mol.<sup>3d</sup> The computed Fe–S shortening due to NH–S<sup>-</sup> hydrogen bonding (by 0.029 Å) matches the experimental data of Suzuki et al.<sup>6</sup> Assuming additivity of the effects, the Fe–S bond length under conditions of hydrogen bonding and medium polarization is ca. 2.44–2.42 Å.

Scheme 2 shows spin density and charge distribution data of the lowest states of compound I under different conditions. The  $A_{2u}$  species is a triradicaloid<sup>3d,e</sup> with three unpaired electrons, two of which are localized in the FeO moiety and maintain a triplet situation, while the third electron with either spin up (positive spin density) or spin down (negative spin density) is distributed over the thiolate and porphyrin ligand. In the bare system, the third spin is spread over both ligands but located more on the thiolate.<sup>3a</sup> With hydrogen bonding, there is a dramatic shift of the spin density which is now localized more heavily on the porphyrin. An enhanced localization is exerted by the polarization effect, and already a small dielectric constant of 5.7 achieves the effect. Similar information is provided by the group charges ( $Q$ ) which show that an initially negative porphyrin (Por) becomes positively charged and an almost neutral thiolate for the bare system becomes negatively charged under the influence of NH–S<sup>-</sup> bonding and medium polarization. Thus, *the environmental factors change compound I from a thiolate radical species, PorFe<sup>(IV)</sup>(O)SH<sup>\*</sup>, to a porphyrin cation radical species, Por<sup>+</sup>Fe<sup>(IV)</sup>(O)SH<sup>-</sup>.*

The present theoretical results remove previous disparities with experimental data. Thus, B3LYP results for the bare system give an Fe–S distance in the range of 2.6–2.7 Å, and indicate that the species has a dominant thiolate-radical character, PorFe<sup>(IV)</sup>(O)SR<sup>\*</sup>.<sup>3a,d,e</sup> In contrast, now with hydrogen bonding accounted for and medium polarization, the theoretical Fe–S bond length drops to ca. 2.44–2.42 Å, and the electronic structure is more consistent with a porphyrin cation radical, Por<sup>+</sup>Fe<sup>(IV)</sup>(O)SR,

**Scheme 3.** Resonance Forms and Their Mixing for 1–3

species. The experimentally gauged<sup>1</sup> Fe–S distance is  $2.3 \pm 0.2$  Å. Also, the only known compound I of a thiolate enzyme is that of CPO, and it is reported to be “green” and to possess spectroscopic properties of a Por<sup>+</sup> situation.<sup>7,13</sup> Thus, the environmental effects bring the theoretical description of compound I closer to the that of the experimental species.

All these effects are associated with the redox mesomerism which has been proposed<sup>2</sup> as the factor that distinguishes thiolate-based compound I from its nonthiolate analogues. In this description, the species is a resonance hybrid of two forms **a** and **b** shown in Scheme 3. At infinite separation the more stable form is the neutral pair **a**, while the ion-pair form **b** is considerably higher, and with the LACVP basis set is computed to be larger than 100 kcal/mol depending on the thiolate model used. At shorter distances **b** is stabilized by electrostatic interactions and the gap is significantly smaller. In the bare system, the neutral form **a** is the lowest energy form, and the result of the mixing of the two forms is a compound I species with a dominant thiolate-radical character, PorFe<sup>(IV)</sup>(O)SR<sup>\*</sup>. Hydrogen bonding to the thiolate ligand (NH–S<sup>-</sup>) stabilizes resonance structure **b** and leads to a compound I species with a dominant porphyrin cation radical, Por<sup>+</sup>Fe<sup>(IV)</sup>(O)SR, character. The medium polarization slightly enhances this trend but its major effect is to solvate better the polarizable **b**-like species. In the enzyme’s pocket, the stabilization will be exerted by the protein helix which exposes the positive ends of the polar groups to the iron porphyrin species.<sup>4</sup> Since at infinite Fe–S distance form **a** is still the lowest in energy, the net stabilization of compound I at its equilibrium Fe–S distance is expressed as the bond strengthening effect. Our previous calculations show that the Fe–S bond energy in the bare compound I is 6.9 kcal/mol.<sup>3d</sup> The present estimate of the stabilization due to solvent polarization is about 15 kcal/mol (for  $\epsilon = 5.7$ ). Taken together, the Fe–S bond energy increases to ca. 22 kcal/mol.

In summary, the internal donor capability of the thiolate ligand vis à vis the acceptor property of the Por<sup>+</sup>Fe<sup>(IV)</sup>=O species gives rise to a hybrid nature of compound I. The interactions in the protein pocket are predicted to tighten and strengthen the Fe–S bond and endow the species with a dominant porphyrin cation radical character. These effects are presumably important also in the reactivity patterns of P450 as a mono-oxygenase species.<sup>2,6,7</sup> This intriguing effect may be associated with enzyme specificity modulated by the protein environment.

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