The Iron−Sulfur Bond in Cytochrome *c*

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We present a density functional study on models of the Fe(II)− and Fe(III)−cytochrome *c* prosthetic groups. Our calculations indicate that most of the well-known stability of Fe(II) relative to Fe(III) is due to stronger metal−S (Met 80) σ interactions. The polarization effects due to the protein frame appear not to affect significantly the Fe−S bonding.

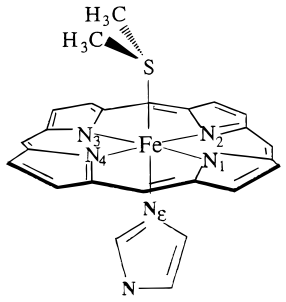
Introduction

Cytochrome *c* is a heme−protein mediating single electron transfer in the mitochondrial electron-transport chain using the Fe(II)−Fe(III) redox pair.[1] The two redox forms, while structurally similar,[2−4] differ dramatically in their thermodynamical stability. Indeed, the reduced protein is far more stable toward unfolding, as indicated by the large differences in free energies of unfolding[5−8] ($\approx$−10 kcal/mol for the horse and yeast cytochrome *c*[8]). Particularly striking is the difference in stability and reactivity exhibited by the Fe−S (Met 80) bond, which is believed to play a major role in the high redox potential of the protein:[9] in ferric cytochrome *c*, the addition of denaturants such as guanidium hydrochloride causes the breaking of the Fe−S bond and the consequent unfolding of the oxidized species, while the reduced species is stable under the same conditions.[7,8,10,11] Furthermore, while the Fe(II)−S bond is stable over a wide pH range (4−12), the Fe(III)−S bond breaks already at pH 9.3.[12] The strength of the Fe(II)−S bond is also demonstrated by the differences in the binding of ligands such as CO, CN^-, and N_3^-.[13]

The surprising affinity of divalent sulfur for Fe(II) is an object of debate: it may arise from the *softness* of the methionine sulfur,[14] which would favor the binding to the *soft* Fe(II) ion more than to the *hard* Fe(III) ion. As spectroscopic studies suggest,[15] the electron π acceptor character of sulfur[13,16,17] could also play a role: the Fe d−S d back-donation would stabilize Fe(II) relative to Fe(III) because of its larger electron density. However, the existence of a simple σ Fe−S bond has also been proposed.[18]

To assess these proposals and reveal other possible factors playing a critical role in the Fe−S bonding, we have performed a density functional investigation of heme systems on model complexes of the reduced and oxidized cytochrome *c* prosthetic group. We have also estimated to what extent the protein surroundings affect the electronic structure of the iron complex by including its electric field in our calculations. To our knowledge, these issues have not been previously addressed by means of quantum-mechanical methods.

CHART 1



Methodology. Our model of the cytochrome *c* prosthetic group is the FeP−Im−DMS (FeP = iron porphyrin, Im = imidazole, DMS = dimethyl sulfide) complex (Chart 1). Porphyrin substituents (vinyl, cysteines, and propionates) were not included in the calculations, because, while significantly increasing the computational effort, they have been shown not to affect the electronic structure of similar iron−porphyrin complexes.[19,20] The reference structures for our calculations were the ferrous[21] and ferric[22] yeast iso-1 protein, for which the crystal structures are available at 1.23 and 1.8 Å resolution respectively.[23]

Density functional theory calculations were performed using the Car−Parrinello method[24] on the [Fe(II)P−Im−DMS] and [Fe(III)P−Im−DMS]$^+$ complexes. The same computational procedure has already been applied with success in the modeling of the prosthetic group in myoglobin.[19,20,25] Full geometry optimizations were carried out without symmetry constraints. In the resulting structures, DMS turns out to be compatible with the positions of the C(β)−S−C(γ) atoms of Met 80 in oxidized and reduced yeast protein.[21−23] The Kohn−Sham orbitals were expanded in a plane wave basis set up to an energy cutoff of 70 Ry. The complexes were enclosed in a supercell of sides 14.5 × 16.0 × 14.5 (Fe(II)) Å^3 and 17.0 × 19.0 × 18.0 Å^3 (Fe(III)). The ground states of oxidized and reduced forms were found to be doublet and singlet spin states ($S = {}^1/_2$ and S = 0), respectively, consistent with experimental data.[1] Spin states of

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TABLE 1: Calculated and Experimental (in brackets) Properties of [Fe(II)P−Im−DMS] and [Fe(III)P−Im−DMS]$^+$ Complexes[a]

	oxidized	reduced
BE (kcal/mol)	−3.5	−7.5
BO	0.6	0.7
d(Fe−S) (Met 80) (Å)	2.27 [2.43[c]]	2.23 [2.35[b]]
d(Fe−Nϵ) (His 18) (Å)	2.04 [2.01]	2.04 [1.99]
d(Fe−N) (P) (Å)	2.01(2) [(2.00(3)]	2.00(0) [1.99(1)]

[a] Standard deviations are reported in parentheses. Explanations of the symbols in the text. [b] From ref 22. [c] From ref 21.

higher multiplicities, [Fe(II)P−Im−DMS] ($S = 1$) and [Fe(III)P−Im−DMS]$^+$ ($S = {}^3/_2$), turned out to be rather higher in energy, namely 20.2 and 17.8 kcal/mol, respectively, at the ground state geometries. Calculations on the isolated fragments ([Fe(II)P−Im], [Fe(III)P−Im]$^+$, and DMS) were done in their ground states, which turned out to be $S = 1$,[20] $S = {}^3/_2$, and $S = 0$, respectively. Some calculations included the electrostatic potential of the yeast iso-1 protein in both redox forms.[26] The potential Φ (in atomic units) at the position r was calculated as:

$$\Phi(r) = \sum_i \frac{q_i}{(r_i - r)}$$

where q_i are the RESP[27,28] atomic point charges at the position r_i.[29]

Results

To determine the stability of the Fe−S bond in the two redox states, we calculate the binding energies (BE's) as the difference between the energy of the optimized complex and the energies of the isolated fragments (DMS, [Fe(II)P−Im], [Fe(III)P−Im]$^+$). Table 1 indicates that Fe(II)−S and Fe(III)−S bonds are stable upon dissociation and that the reduced state has the strongest binding. Consistently, the electronic affinity calculated at optimized geometry of the ferrous state turns out to be negative (−128 kcal/mol).

Analogously to previous quantum chemical calculations on other heme systems,[19,20] the porphyrin ring turns out to be essentially flat in our optimized structures. Therefore, the Cys−

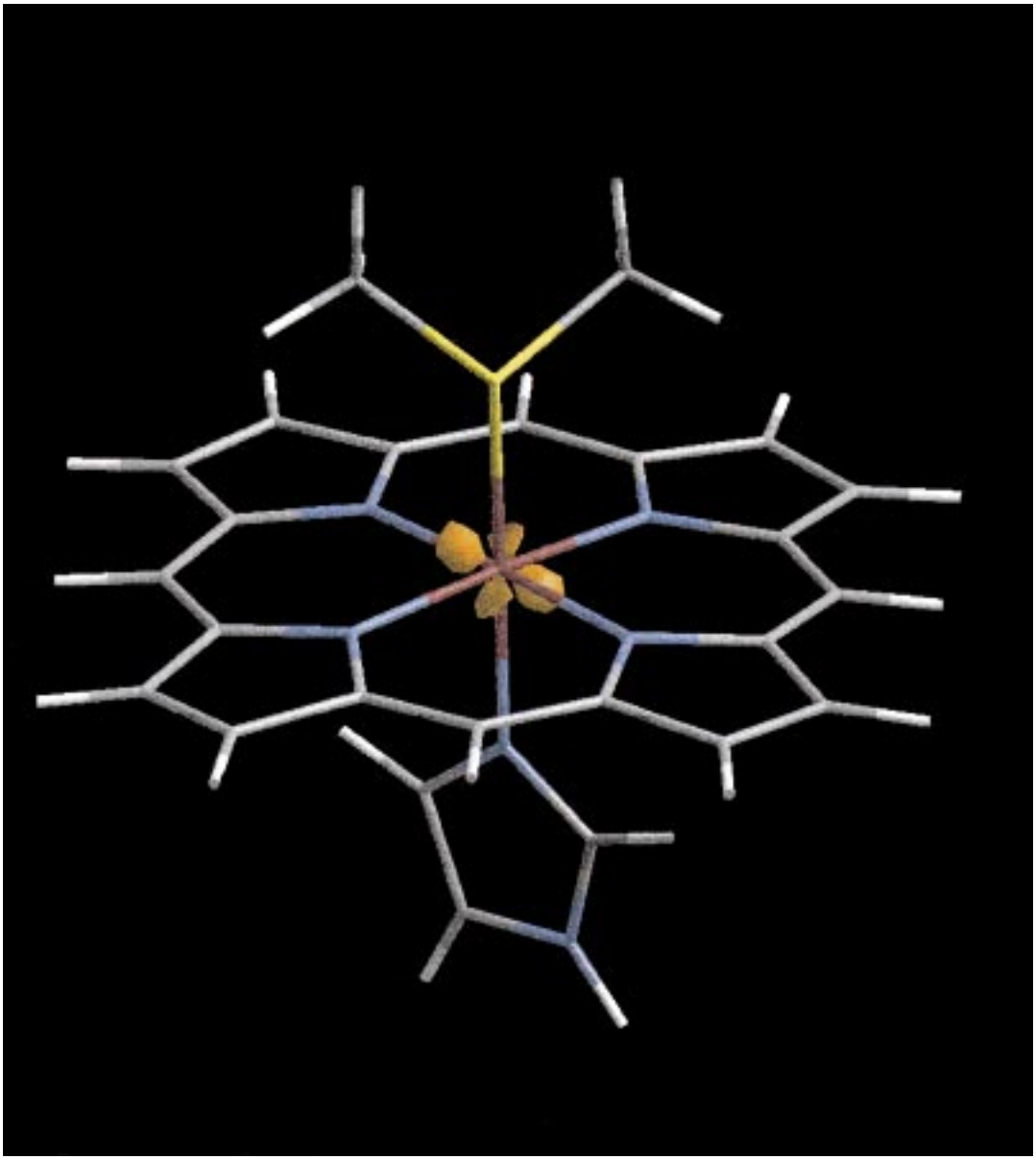

Figure 1. Isodensity contour (0.05 au) of the electronic density difference between reduced and oxidized forms. The geometry is that of the optimized reduced form.

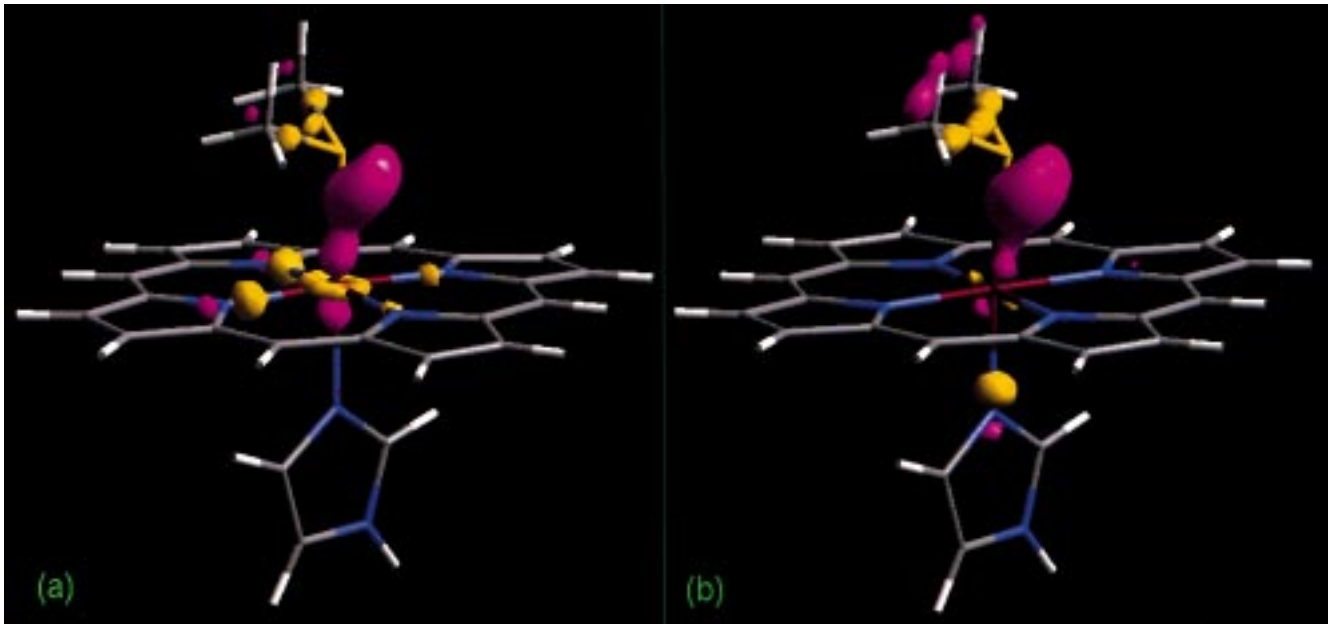

Figure 2. Isodensity contours of inner MO's of (a) reduced and (b) oxidized forms. In (b), only one spin−orbital is shown. Yellow, 13 au; magenta, −13 au.

vinyl link with the protein, as well as the rest of the protein itself, is likely to be the only factor responsible for the well-known distortion from planarity of the heme group in cytochrome *c*.[1]

Our calculations reproduce the experimental finding that the Fe−S distance is shorter in the reduced state[2,21,22] (Table 1). Hence, the higher affinity of Met 80 sulfur for heme Fe(II) is, at least partly, an intrinsic property of the prosthetic group. Our calculated Fe−S bond lengths are somehow shorter than those of the starting X-ray structures of oxidized and reduced yeast iso-1 proteins. However, additional calculations at various Fe−S distances show that the energy hardly changes with respect to a small elongation of the Fe−S bond up to the experimental values (less than 0.5 kcal/0.1 Å). Thus the difference between theory and experiment can be ascribed in part to the effect of the protein. Table 1 and Table 1S in the Supporting Information also show that other features of the Fe−S coordination polyhedra are well reproduced by our calculations.

We now describe the rearrangement of the electronic density ρ upon uptake of an electron by calculating the electronic density difference between reduced and oxidized species at fixed geometry. We used as reference geometry both the oxidized and reduced forms and found the results to be insensitive to such choices. As shown in Figure 1, the extra electron turns out to be entirely localized on the iron ion and polarization effects on the methionine sulfur are absent. These findings are completely consistent with an electrostatic study based on mechanical modeling of cytochrome *c*, which indicates that the loss of electron density on oxidation is essentially localized only on the iron ion.[30]

The Fe d level splitting in the complexes investigated here is characteristic of a low-spin iron ion in octahedral ligand field: the three highest occupied molecular orbitals (MO's) are essentially the d_{xz}, d_{yz}, d_{xy} orbitals, while the d_{z^2} and $d_{x^2-y^2}$ orbitals lie among the unoccupied MO's. Analysis of the atomic orbital character of lower-lying MO's and the Mayer bond order[31] (See Table 1) allows the orbitals that exhibit the strongest Fe−S interaction in the two species to be identified.

The most significant contributions arise from the iron d orbitals and the sulfur lone pairs.

In the reduced species, the lone pair directed along the Fe−S bond mixes strongly with the iron d_{z^2} orbital, forming a σ Fe−S MO (Figure 2a). The iron d_{z^2} hybridizes also with the nitrogen donor atoms.

In the oxidized species, competing factors may affect the Fe d_{z^2} interaction with the sulfur lone pair directed along the Fe−S bond. On the one hand, oxidation shifts down the energy of the iron d levels, which in turn may *increase* the Fe d_{z^2}−S overlap. On the other hand, the Fe d orbitals are less diffuse and Fe−S distance is longer than in the reduced state, which may cause a *decrease* of the Fe d_{z^2}−S interaction. Analysis of the electronic structure shows that the latter factors predominate, as this iron d_{z^2}−S interaction is almost absent in the oxidized species.

The only other MO exhibiting significant Fe−S interactions involves the other S lone pair forming a π bond with the d_{yz} iron orbital; thus it is much higher in energy and contributes less to the bond strength than the other. The characteristics of this latter MO's are very similar in both the reduced and oxidized form. Figure 2b shows this π MO for the oxidized species as an example.

We conclude that the global effect of both σ and π MO's is that of favoring the Fe(II)−S bond over the Fe(III)−S one.

Environment effects might affect significantly the F−S bonding in cytochrome *c*,[1] as sulfur is a very polarizable atom. To provide a qualitative estimation of the polarization due to the surrounding protein, we have also carried out calculations on both redox forms in which the electrostatic potential of the yeast iso-1 protein frame is included.[26] The change in Fe−S chemical bonding is conveniently analyzed in terms of maximally localized Wannier functions, which efficiently visualize chemical concepts such as lone pairs and localized covalent bonds.[32]

Table 2 reports the locations of the Wannier centers of the Fe−X bond (X = axial donor (N or S)), which represent the donor's lone pairs pointing toward the metal ion. The table shows that the effects on the two bonds with the axial ligands

TABLE 2: Fe−X (X = Axial Donor (N or S)) Bond: Distance (Å) between the Wannier Function Centers and the Fe or X Atoms in the Absence and in the Presence of the Electric Field of the Yeast Iso-1 Protein[26] [a]

	isolated system		protein potential	
	Fe	X	Fe	X
reduced form				
Fe−S (Met 80)	1.64	0.72	1.62	0.74
Fe−Nϵ (His 18)	1.47	0.53	1.46	0.53
oxidized form				
Fe−S (Met 80)	1.67(5)	0.77(4)	1.66(5)	0.78(4)
Fe−Nϵ (His 18)	1.48(3)	0.53(3)	1.49(3)	0.52(2)

[a] For the oxidized form, the average values of the spin-up and spin-down centers are reported.

are actually similar and rather small in both oxidized and reduced forms, as the protein field does not cause significant shifts of the donors' lone pairs. Similar conclusions can be drawn for the Fe−porphyrin donor atoms (data not shown).

Discussion

In agreement with experimental evidence, our calculations indicate that the reduced form of the cytochrome *c* prosthetic group is lower in energy than that of the oxidized form. This indicates that Coulomb interactions, which are expected to favor the ferric species, do not play an important role in the stability of the Fe−S bond. However, since the calculated difference in binding energy is substantially smaller (−4.0 kcal/mol) than the observed differences in free energy of folding of the protein, the stabilization of the reduced species is only partly due to the properties of this bond. Other factors, such as the different level of hydration[33] and the small but significant structural rearrangements of the entire protein on passing from the oxidized to the reduced form,[2,3] are expected to play an important role.[34]

Our calculations provide no evidence for the postulated role of the π acceptor ability of Met 80 sulfur in the stabilization of the reduced state:[13,16,17] neither is the electron density of the Fe−S bond affected on passing from the oxidized to the reduced form (Figure 1)[30] nor is evidence found for Fe d−S d orbital interactions.[13] Instead, the sulfur appears to act as σ electron donor (Figure 2).[18] Our calculations show that the net σ Fe−S interaction is more pronounced in the case of the ferrous species. Therefore, we conclude that a crucial factor enhancing the stability of the reduced species is the larger Fe−S hybridization in the reduced species, which corresponds to the "soft" character of ligand and metal ion.[14]

It is well-known that methionine has a very poor affinity for iron.[35] Consistently, in our model complexes, the Fe−S bond is remarkably weak: other ligands commonly binding to iron in heme proteins, such as such as O_2, NO, histidine residues, and CO, exhibit binding energies ranging from −15 to −35 kcal/mol with the same computational setup.[20]

Because sulfur is a highly polarizable atom, the protein electrostatic field might affect the Fe−S bond considerably. However, our calculations suggest that the polarization of the sulfur's electron lone pair along the Fe−S bond is minimal. More sophisticated models of the electrostatic potential, which for example includes the electron polarizabilities of the atoms of the protein and of the solvent, are expected to reduce the electric field at the prosthetic group and hence to give essentially the same picture.

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Supporting Information Available: Table reporting selected structural properties of reduced and oxidized forms of FeP(Im)(DMS) and yeast iso-1 proteins.[21,22] The material is available free of charge via the Internet at http://pubs.acs.org.

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