Structure and Redox Properties of the Protein, Rubredoxin, and Its Ligand and Metal Mutants Studied by Electronic Structure Calculation†

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The changes in the structural and electronic properties accompanying metal ionization of the iron-containing protein, rubredoxin, and of some ligand and metal mutants, have been explored using density functional theory (DFT) calculations of the metal atom coordinated to the four immediate residues. Both isolated and embedded cluster studies have been carried out, the latter using the hybrid quantum mechanics/molecular mechanics (QM/MM) approach. The replacement of a cysteine by a serine residue has a considerable effect on both the electronic and geometric structure of the core, which can be qualitatively understood on the basis of the isolated cluster studies. The modulation of these properties caused by the protein environment is quite accurately described by the QM/MM calculations. The predicted core geometries are in good accord with both X-ray and EXAFS data, and the changes in the redox potentials are predicted, at least semiquantitatively, by considering only the core part of the protein.

Introduction

An understanding of the structure−function relationship of electron-transfer proteins may be sought in terms of the electronic structure of the core involved in electron transfer and how this is modified by the protein environment.[1−4] Rubredoxins (Rd), which contain a single iron site where the metal atom is coordinated to four cysteine residues, are the simplest iron−sulfur electron-transfer proteins, and their structure−function relationships have been extensively studied.[5−8] The electronic properties of the iron−sulfur core have been probed experimentally using electron detachment spectroscopy of model anionic clusters, as well as by a variety of spectroscopic techniques such as photoelectron spectroscopy applied to more accessible model compounds.[9−12] These measurements, combined with complementary computational studies, have resulted in an understanding of both the nature of the iron−sulfur bonding and the energetics of the electron ionization process, quantities which can be readily calculated for such small molecules. As far as the proteins themselves are concerned, there are structural and redox data for both the native Rd itself and for a number of mutants.[13−20] In particular, the effect on both the structure and the redox properties of mutating a cysteine ligand to serine has been studied, when different effects have been found for mutation of the surface and buried ligands.[14−16] There have also been some structural studies of metal-substituted forms of Rd in which the iron atom has been replaced by Co, Ni, Ga, Cd, or Hg.[13] On the theoretical side, there has been a quantum chemical study of a number of these metal-substituted analogues, in which the tetrahedral core structure itself has been studied.[17]

The structure and reactivity of proteins themselves are now quite regularly studied using so-called hybrid or multilevel methods, in which the reactive part of the protein is described using an appropriate "high" level of quantum mechanics (QM), while the protein environment is modeled using a lower level of theory, often a molecular mechanical (MM) force field.[21,22] We here describe electronic structure calculations of model clusters involving the metal atom and a variety of different ligands in the first coordination shell, to probe the basic bonding in the two oxidation states, followed by quantum mechanics/molecular mechanics (QM/MM) calculations of Rd itself, and of a series of proteins in which either the central iron atom or the cysteine ligands have been mutated.

Theoretical Basis

We have studied both the isolated active site of each protein, using density functional theory (DFT), and the protein itself, using a hybrid or multilevel model combining a DFT description of the active site, with a MM description of the remainder of the protein. In all QM calculations using DFT, there is the question as to which functional and basis set to employ. This problem can be particularly acute for molecules containing transition metals where there are a number of possible spin states, since the so-called hybrid class of functional (e.g., B3LYP) have varying amounts of explicit exchange which may favor high spin states. It is not generally possible to assess the accuracy of different functionals by using high-level ab initio calculations (e.g., CCSD(T)) since these calculations are often not feasible for moderately sized molecules containing transition metals. We have thus chosen to use the measured values of the vertical and adiabatic electron detachment energies (VDE, ADE) for the high-spin species, $[FeCl_3]^{1-/0}$, $[FeBr_3]^{1-/0}$, and $[Fe(SCH_3)_3]^{1-/0}$, to compare the commonly used B3LYP functional with the BP86 functional, the latter also being quite widely used to study transition metal complexes[23,24] and to compare different basis sets (Table 1). At the 6-31G* level, we see there are considerable differences between the ionization energies for the two functionals, with those from BP86 being smaller than those from B3LYP, presumably due to the lack of explicit exchange in the former. When compared with experiment, the B3LYP values are in very good agreement. These differences between the performance of the two functionals also extend to the reorga-

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TABLE 1: Structures (Å) and Energies (eV) of Rubredoxin Analogues

	$[FeCl_3]^{1-/0}$			$[FeBr_3]^{1-/0}$			$[Fe(SCH_3)_3]^{1-/0}$		
	expt[a]	reduced	oxidized	expt[a]	reduced	oxidized	expt[b]	reduced	oxidized
				B3LYP/6-31G*					
Fe−X		2.24	2.14		2.38	2.28		2.31	2.21
VDE	4.36	4.24		4.42	4.35		3.08	2.94	
ADE	4.10	4.00		4.26	4.17		2.80	2.70	
λ_{oxi}	0.26	0.24		0.16	0.18		0.28	0.24	
				BP86/6-31G*					
Fe−X		2.22	2.14		2.33	2.26		2.26	2.20
VDE	4.36	3.61		4.42	3.34		3.08	2.14	
ADE	4.10	3.47		4.26	3.25		2.80	2.03	
λ_{oxi}	0.26	0.14		0.16	0.09		0.28	0.11	
				B3LYP/TZVP (Fe): 6-311++G**					
Fe−X		2.27	2.16		2.41	2.30		2.33	2.23
VDE	4.36	4.56		4.42	4.47		3.08	2.99	
ADE	4.10	4.31		4.26	4.25		2.80	2.79	
λ_{oxi}	0.26	0.25		0.16	0.22		0.28	0.20	

[a] Reference 35. [b] Reference 12.

TABLE 2: QM (bold) and MM Residues Optimized in QM/MM Calculations

proteins	amino acid residues
Fe−Rd	Thr5, **Cys6**, Thr7, Val8, **Cys9**, Gly10, Tyr11, Ile12, Val38, **Cys39**, Pro40, Leu41, **Cys42**, Gly43, Val44, Gly45, **Fe54**
Cys6Ser	Thr5, **Ser6**, Thr7, Val8, **Cys9**, Gly10, Tyr11, Ile12, Val38, **Cys39**, Pro40, Leu41, **Cys42**, Gly43, Val44, Gly45, **Fe54**
Cys42Ser	Thr5, **Cys6**, Thr7, Val8, **Cys9**, Gly10, Tyr11, Ile12, Val38, **Cys39**, Pro40, Leu41, **Ser42**, Gly43, Val44, Gly45, **Fe54**
Co−Rd	Thr5, **Cys6**, Thr7, Val8, **Cys9**, Gly10, Tyr11, Ile12, Val38, **Cys39**, Pro40, Leu41, **Cys42**, Gly43, Val44, Gly45, **Co54**
Ni−Rd	Thr5, **Cys6**, Thr7, Val8, **Cys9**, Gly10, Tyr11, Ile12, Val38,**Cys39**, Pro40, Leu41, **Cys42**, Gly43, Val44, Gly45, **Ni54**

nization energy (λ_{oxi}, the difference between the VDE and ADE values), a quantity central to the Marcus theory of electron transfer.[25] As far as basis sets are concerned, we find that enlarging the basis to TZVP for the iron, and 6-311++G** for the remaining atoms, does little to improve the correlation with the experimental data (Table 1). We thus believe that it is appropriate to use the B3LYP/6-31G* level of theory.

To describe the effect of the protein environment on the QM core, QM/MM calculations were carried out employing the ONIOM scheme as implemented in GAUSSIAN03[26,27] using the AMBER force field[28] for the MM region. Within ONIOM, we use the electronic embedding scheme in which the QM region is polarized by the MM point charges.[29] Our computational procedure is to take the crystal structure of the oxidized form of the native protein (PDB code: 1IRO), solvate it with a large number (3900) of TIP3P[30] water molecules, and relax the protein using the AMBER force field. To give an initial structure of the serine and metal mutants, this structure was taken and the appropriate mutation carried out. Thus, any structural changes which occur upon mutation of either cysteine or the metal atom are dependent only upon our calculations, rather than upon experimental data.

In the QM/MM calculations, the QM region consisted of the metal atom and the four ligands coordinated to it. Thus, for Rd, the $[Fe(SCH_3)_4]$ cluster was taken as the QM region and was described at the DFT level of theory (B3LYP/6-31G*). Here, the cysteines are partitioned between the C^{β} and C^{α} bonds (SCH_3). A similar partitioning was used for the mutated Rd where the serine is partitioned between the C^{β} and C^{α} bonds (OCH_3). All the atoms in the QM region, together with the neighboring MM residues, were optimized, which includes all the six hydrogen bonds to the active site, while the remainder of the protein and solvating waters were held fixed (Table 2).

We wish to predict the redox potentials of a series of Rd proteins. However, the calculation of the absolute value of the redox potential of proteins presents formidable difficulties, which largely center around the sampling of the many possible configurations of the protein and the evaluation of the associated entropy. For this reason, it is usual to compute the *differences* in redox potential between similar proteins,[31,32] using simplified models in the hope that such entropic terms, and perhaps other effects, such as the differential solvation of the two oxidation states, will effectively cancel. In one attempt to predict the absolute redox potential of the blue copper protein, plastocyanin, experimental data were used to estimate the entropic contributions to the redox potential.[33] The problem is further accentuated by the quite small differences in redox potential, of the order of 100 meV, which we are trying to understand.[34] In this paper, we are mainly concerned with active site structures and we wish to see if the trends in the redox potentials can be predicted in terms of the measured and predicted structural changes at the active site.

Results and Discussion

(a) Isolated Cluster. We first discuss the results of our studies on the isolated clusters, focusing on the effects of mutating either the cysteine groups or the central metal atom. In Table 3,we see the effect on both the structure and ionization energies of progressively replacing methyl thiols by methoxy groups. The replacement of a "soft" ligand ($-SCH_3$) by the "harder" $-OCH_3$ ligand causes a progressive increase in the Fe−S lengths for both the reduced and oxidized forms. We see that for these species which possess a double negative charge, the VDE and ADE are strongly negative, both values decreasing with the degree of replacement of $-SCH_3$ by $-OCH_3$. Changes in the VDE values can be considered to arise from two effects: first, changes in the ionization energy when the remaining electrons are not allowed to relax (as in Koopmans' theorem (KT) approximation), with the orbital relaxation energy being the second contribution (Table 4). At the KT level, ionization occurs from an essentially metal localized MO, giving a positive ionization energy for all four species, whose value decreases progressively upon substitution of sulfur by oxygen. We can ascribe this effect to the stabilization of the metal charge, which increases across the series, by the strongly electronegative

TABLE 3: Structures (Å) and Energies (eV) of Rd Mutant Analogues[a]

	$[Fe(SCH_3)_4]^{2-/1-}$		$[Fe(SCH_3)_3(OCH_3)]^{2-/1-}$		$[Fe(SCH_3)_2(OCH_3)_2]^{2-/1-}$		$[Fe(SCH_3)(OCH_3)_3]^{2-/1-}$		$[Fe(OCH_3)_4]^{2-/1-}$	
	reduced	oxidized	reduced	oxidized	reduced	oxidized	reduced	oxidized	reduced	oxidized
					Isolated					
Fe−S	2.43	2.32	2.45	2.33	2.49	2.35	2.50	2.37		
Fe−O			1.93	1.83	1.95	1.85	1.98	1.86	2.00	1.87
					Solvated ($\epsilon = 4.9$)					
Fe−S	2.40	2.32	2.43	2.33	2.47	2.35	2.49	2.38		
Fe−O			1.91	1.82	1.93	1.84	1.98	1.85	1.99	1.87
					Isolated					
VDE	−1.90		−2.21		−2.59		−3.07		−3.43	
ADE	−2.17		−2.54		−3.12		−3.70		−4.27	
λ_{oxi}	0.27		0.33		0.53		0.63		0.84	
					Solvated ($\epsilon = 4.9$)					
VDE	2.33		1.99		1.66		1.13		0.68	
ADE	2.17		1.73		1.22		0.58		−0.42	
λ_{oxi}	0.16		0.26		0.44		0.55		1.10	

[a] B3LYP/6-31G*.

TABLE 4: Orbital Relaxation in the Oxidized States of Analogues of Rd and Mutants[a]

		Mulliken charge			VDE	
	atom	reduced	oxidized (KT)	oxidized (SCF)	KT	SCF
$[Fe(SCH_3)_4]^{2-/1-}$	Fe	+0.665	+1.578	+0.725	+4.11	−1.90
	S	−0.522	−0.509	−0.369		
$[Fe(SCH_3)_3OCH_3]^{2-/1-}$	Fe	+0.750	+1.630	+0.866	+3.30	−2.21
	S	−0.559	−0.533	−0.403		
	O	−0.671	−0.665	−0.635		
$[Fe(SCH_3)_2(OCH_3)_2]^{2-/1-}$	Fe	+0.824	+1.709	+1.026	+2.70	−2.59
	S	−0.596	−0.563	−0.441		
	O	−0.687	−0.679	−0.653		
$[Fe(SCH_3)(OCH_3)_3]^{2-/1-}$	Fe	+0.827	+1.747	+1.104	+2.49	−3.07
	S	−0.626	−0.610	−0.466		
	O	−0.685	−0.673	−0.658		
$[Fe(OCH_3)_4]^{2-/1-}$	Fe	+0.784	+1.739	+1.151	+1.84	−3.43
	O	−0.689	−0.682	−0.663		

[a] The atom charges (Mulliken) are given for the reduced state and for the ionized states with (SCF) and without (KT) orbital relaxation. The corresponding VDEs (eV) are also given.

oxygen atom(s). Following ionization, electron relaxation occurs involving electron transfer from the ligands to the hole on the iron atom, resulting in a decrease of the VDE from the KT values. Such electron transfer is mainly from the thiol groups and is sufficiently large to result in negative ionization energies. The degree of electron transfer decreases as the number of methoxy groups increases, thus reducing the lowering of the ionization energies found at the KT level, due to methoxy substitution. The large relaxation energy shown by the tetrathiolate results in a quite small bond length change (0.11 Å) upon ionization and correspondingly small values for the reorganization energy (λ_{oxi}) (0.27 eV). The reduction in the relaxation energy upon methoxy substitution leads to somewhat larger reductions in the bond lengths upon ionization (>0.11 Å), together with larger reorganization energies (>0.5 eV) (Table 3).

There are some interesting effects associated with the inclusion of solvation via the COSMO continuum model (Table 3), which we have studied using the dielectric constant of chloroform ($\epsilon = 4.9$). The continuum preferentially stabilizes the reduced (2−) species resulting in positive ionization energies for all the species studied. Also, the reduction in bond lengths is smaller in solution which leads to a smaller reorganization energy for all the species compared to the gas phase. For tetrathiolate, the reorganization energy is 0.16 eV (Fe−S bond length difference, 0.08 Å) compared to the gas-phase value of 0.27 eV (Fe−S bond length difference, 0.11 Å).

(b) Protein Studies. In Rd, there are two different environments for the cysteine residues which are coordinated to the central iron atom. Cys6 and Cys39 are buried within the protein, while Cys9 and Cys42 are at the protein surface. The former internal cysteine residues each have two hydrogen bonds to nearby residues. Cys6 is hydrogen bonded both to Val8 and to the surface Cys9. Cys39 is similarly hydrogen bonded to Val41 and Cys42. Each of the two surface cysteine residues possess a single hydrogen bond, Cys9 to Tyr11 and Cys42 to Ala44. This difference in hydrogen bonds between the two sets of cysteine residues leads to an elongated Fe−S bond length for the buried cysteine bond (2.29 Å) to the iron than the surface cysteine ligand (2.24 Å). Mutation studies show that these hydrogen bonds are critical in tuning the redox potential, when mutation of Ala44 by Val44 reduces the value by ∼50 meV. Mutation of the cysteine residues by serine also changes the value of the redox potential, replacing the surface Cys42 by Ser42 and leading to a decrease in redox potential of ∼200 meV;[16] while mutating a buried cysteine residue (Cys6) by serine decreases the redox potential by only ∼100 meV.[15] A crystal structure is available for the Cys42Ser mutant but not for the Cys6Ser mutant. Such a reduction in the redox potential upon replacement of a methyl thiol group by methoxy is indeed shown in the VDEs of both the isolated and solvated (continuum) cluster models which we have already described, although the predicted reduction (∼400 meV) is considerably greater than the experimental values. These cluster models cannot, of course, distinguish between the two different cysteines in the protein.

We have therefore carried out QM/MM calculations on both native Rd and the mutants Cys6Ser and Cys42Ser. The

TABLE 5: Structures (Å) and Energies (eV) of Embedded (QM/MM) Clusters of Rd and Serine Mutants[a]

	native				Cys6Ser			Cys42Ser			
	X-ray [b]	EXAFS[b]	reduced	oxidized	EXAFS[b]	reduced	oxidized	X-ray[a]	EXAFS[b]	reduced	oxidized
$Fe-S_{cys6\ and\ cys39}$	2.29	2.33 (2.27)[c]	2.43	2.34	2.32 (2.28)[c]	2.50	2.40	2.34	2.36 (2.29)[c]	2.48	2.37
$Fe-S_{cys9\ and\ cys42}$	2.24		2.35	2.27		2.39	2.30	2.37		2.39	2.29
$Fe-O_{ser6}$					2.09 (1.87)[c]	1.91	1.84				
$Fe-O_{ser42}$								1.82	1.93 (1.84)[c]	1.91	1.82
VDE			−1.95			−2.27				−2.35	
ADE			−2.01			−2.38				−2.47	
λ_{oxi}			0.06			0.11				0.12	

[a] B3LYP/6-31G*. [b] X-ray and EXAFS values are taken from ref 16. [c] Values in parentheses denote oxidized state for the Fe−X bond lengths.

TABLE 6: Predicted Hydrogen Bonds (Å) in Rd and Its Serine Mutants Using QM/MM Method[a]

	native			Cys6Ser		Cys42Ser		
residues	X−ray[b]	reduced	oxidized	reduced	oxidized	X−ray[b]	reduced	oxidized
Val8N−SCys6	3.68	3.47	3.78	3.80	4.38	3.70	3.58	3.98
Cys9N−SCys6	3.65	3.50	3.83	3.91	4.32	3.71	3.73	4.10
Tyr11N−SCys9	3.49	3.29	3.40	3.23	3.32	3.42	3.27	3.33
Val41N−SCys39	3.54	3.30	3.53	3.72	3.54	3.54	3.32	3.51
Cys42S−SCys39	3.58	3.52	3.64	3.81	3.65	3.56	3.48	3.59
Ala44N−SCys42	3.84	4.35	3.57	3.89	3.46	4.00	4.12	3.83

[a] B3LYP/6-31G*. [b] Oxidized structures (ref 16).

computed structures and the ionization energies (VDE, ADE, and reorganization energies) are shown in Table 5. We first discuss the structure of the native Rd. The experimental Fe−S bond lengths for the buried cysteine ligands are greater than those for the surface residues, due to the greater number of hydrogen bonds to the former. This effect is reproduced both qualitatively and quantitatively by the QM/MM calculations. The entatic effect of the protein environment is also evident from the calculations, where the changes in these bond lengths upon ionization are somewhat smaller than the gas-phase values. The QM/MM calculations predict changes upon substitution of cysteine by serine that are qualitatively the same as those we found from the isolated cluster calculations. Thus, there is an increase in all of the Fe−S bond lengths upon mutation of a single cysteine residue by serine, by up to 0.07 Å. This effect is also seen experimentally in both X-ray and extended X-ray absorption fine structure (EXAFS) data for the Cys42Ser mutant (Table 5).[16] EXAFS data gives an average increase in the Fe−S bond lengths of 0.02 Å for both the oxidized and reduced species, in good agreement with our calculations on this particular mutant. On the other hand, X-ray data for the oxidized species gives a somewhat larger increase (0.13 Å) in the bond length to the remaining surface cysteine. These changes in the Fe−S distances are due to changes in the cysteine environments for which there is good correlation between our predicted and the X-ray structures (Table 6). The QM/MM calculations also yield Fe−O bond lengths which are in good agreement with both X-ray and EXAFS data for the oxidized and reduced forms of the Cys42Ser mutant. In contrast to the Fe−S distances, we note there is essentially no change in the Fe−O distance between the isolated and embedded cluster models. The changes in the iron−sulfur bond lengths upon ionization are reflected in the reorganization energies. For the native protein, this value (<0.1 eV) is considerably less than that for both the isolated and solvated cluster. Compared to the native protein, we predict somewhat greater reductions in the metal−ligand bond lengths for the serine mutants upon ionization. This results in a small, but significant, increase in the reorganization energy to close to 0.1 eV, a value still well below the value for the isolated cluster.

Although the success of ab initio and DFT calculations in predicting the structures of gas-phase molecules is well established, the same cannot be said for the use of QM/MM methods to predict protein structures. This due in some measure to the lack of protein structures at atomic resolution. However, increasingly accurate protein structures are becoming available which can be used to assess the quality of QM/MM predictions.[31] In this context, it is useful to evaluate the energy difference between the core structure predicted by QM/MM methods and that measured experimentally. We have done this comparison for Rd itself and for the Cys42Ser mutant and find that the optimized structures are lower in energy than the experimental ones but only by 2.6 and 2.4 kcal mol^{-1} for the native and mutant proteins, respectively. The corresponding values are naturally larger when compared with the isolated clusters optimized without the constraints of the protein, with the X-ray structures, being 5.8 kcal mol^{-1} for both proteins.

TABLE 7: Redox Potentials (meV) of Serine Mutants Relative to Native Rd

protein	expt [a]	isolated cluster	QM/MM
Cys6Ser	−93	−370	−370
Cys42Ser	−196		−460

[a] Reference 15.

Prediction of Relative Redox Potentials

We have previously discussed the difficulties of predicting the redox potentials of proteins and in identifying the origin of any small changes in the value between closely related structures.[31] An understanding of these differences would naturally help in the design of particular mutants with the desired redox properties. A strong contributor to the variation in the redox potential is the change in the geometric and electronic properties of the metal core structure, which we have addressed in this paper. Bearing in mind the limitations of our approach, we may estimate the relative redox potentials of the three Rd molecules studied here, by comparing the adiabatic electron detachment energies of the optimized QM clusters. These values are given in Table 7. We see that the reduction in the redox potentials upon serine mutation is somewhat overestimated (by ∼250 meV), but the difference in the values for the two mutants is given to high accuracy.

TABLE 8: Predicted Structures (Å) and Energies (eV) of Isolated and Embedded Clusters of Metal Mutated Rd[a]

	X = Fe			X = Co			X = Ni		
	X−ray[b]	reduced	oxidized	X−ray[b]	reduced	oxidized	X−ray[b]	reduced	oxidized
				Isolated Cluster					
X−S		2.43	2.32		2.40	2.26		2.38	2.26
VDE		−1.90			−1.27			−1.42	
ADE		−2.17			−1.72			−1.68	
λ_{oxi}		0.27			0.45			0.26	
				Embedded Cluster					
$X-S_{cys6\ and\ cys39}$	2.29	2.43	2.34	2.33	2.41	2.29	2.37	2.38	2.29
$X-S_{cys9\ and\ cys42}$	2.24	2.35	2.27	2.25	2.37	2.22	2.26	2.33	2.22
VDE		−1.95			−1.41			−1.52	
ADE		−2.01			−1.62			−1.57	
λ_{oxi}		0.06			0.21			0.05	

[a] B3LYP/6-31G*. [b] Oxidized structure for the native protein;[16] the reduced structure for both metal mutants.[13]

We have also estimated the redox potentials of the cobalt and nickel mutants of Rd for which X-ray structures have been reported, although we were unable to locate data for the corresponding redox potentials. Yang et al.[35] have reported measurements of the electron detachment energies for the nickel and cobalt halides (MX_4^-, X = Cl, Br), together with the corresponding DFT calculations. However, no experimental data for the analogous methyl thiols have been reported. We have carried out DFT calculations of the VDEs and ADEs of $[M(SCH_3)_4]^{2-}$ (M = Co, Ni), shown in Table 8. These cluster calculations yield detachment energies which, as expected, increase across the periodic table and give reorganization energy for the nickel mutant essentially unchanged from the corresponding value for the iron congener, while that for the cobalt mutant is significantly larger. The metal−sulfur bond lengths from the X-ray structures of the reduced forms of the Co and Ni mutants are very similar to those of Rd itself, with the values for the buried cysteine residues being up to 0.1 Å longer than those for the surface residues. Again, this effect is well reproduced by our QM/MM calculations (Table 8). Although there is very little change in the values of these bond lengths upon metal mutation, we do find that the reorganization energy of cobalt Rd is larger than that for the iron- and nickel-containing proteins as was found for the vacuum models. This could be of significance for the electron-transfer properties of the mutants, but there are no experimental data to test this prediction. The energies of the optimized embedded clusters are very close to those of the crystal structures (1 ROH = CoRd and 1 ROJ = NiRd), by 2.7 and 0.6 kcal mol^{-1}, respectively, which again reflects the value of QM/MM models for structure prediction.

Conclusions

We have here described a computational study of the structure and redox properties of Rd and some mutants, which complements experimental studies. Many of our predictions relate to the structure of the first coordination shell of the metal atom. The calculations on both the reduced and oxidized forms of Rd itself, and on the serine and metal mutants, have started with a single X-ray structure. The good agreement between the calculated and the available experimental structures shows that QM/MM calculations do have good predictive capabilities, but additional X-ray data at atomic resolution would allow a more critical evaluation of the hybrid approach. We have found that the major electronic effects accompanying the mutation of cysteine to serine are evident in isolated cluster studies of the metal core structures. In particular, the reorganization energies increase upon mutation. There is a definite, though quite small, entatic effect[36] of the protein environment, which causes a reduction in the reorganization energy of both the native and mutant proteins. The trends in the redox potential upon mutation are reproduced by considering only the adiabatic electron detachment energies of the optimized metal core structure. We do not speculate as to the origin of the remaining discrepancy in the relative redox potentials, of the order of 6 kcal mol^{-1}. We have carried out a similar study on two metal mutants of rubredoxin and have identified possible differences in their reorganization energies.

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