Ni−Fe Hydrogenases: A Density Functional Theory Study of Active Site Models

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Possible intermediates in the mechanism of dihydrogen activation by the nickel−iron center of Ni−Fe hydrogenases, as proposed by Dole et al. (Dole, F.; Fournel, A.; Magro, V.; Hatchikian, E. C.; Bertrand, P.; Guigliarelli, B. *Biochemistry* **1997**, *36*, 7847−7854), have been investigated using quantum chemical methods. Results obtained on models of the Ni−A, Ni−B, Ni−SI, Ni−C, and Ni−R forms of the enzyme show that (i) despite valence state changes of the nickel ion, the electron density on this metal is very similar in all these forms, (ii) in paramagnetic species, the spin density is mainly localized on the nickel atom and its sulfur ligands, which confirms the diamagnetic nature of the iron ion in the [Ni−Fe] cluster, (iii) in the Ni−C and Ni−R states, a hydrogen atom can bridge the two metal ions without major structural reorganization, apart from a shortening of the Ni−Fe distance, which becomes equal to 2.67 Å. The good agreement between these results and the experimental data obtained on hydrogenases supports the active site structures proposed by Dole et al. for the various states of the enzyme.

Introduction

Hydrogenases are enzymes that catalyze the oxidation of dihydrogen according to the reversible reaction $H_2 \leftrightarrow 2H^+ + 2e^-$ which plays a key role in bacterial metabolism and may have a great relevance in bioenergetics and fuel production. Even if hydrogenases that use only Fe−S centers are known, the most extensively studied are those also containing a Ni center.[1] The X-ray structure of the NiFe-hydrogenase from *Desulfovibrio gigas* has been recently reported;[2] it contains a hetero-dinuclear [Ni−Fe] cluster[3] where a Ni atom coordinated by two terminal cysteine residues is bridged to an Fe atom by two other cysteine residues. The Fe atom is also coordinated by three nonprotein ligands,[4] namely, two CN^- and one CO.[5] In the as-purified form of the enzyme from *D. gigas*,[2] the two metal ions are also bridged by an oxygenic nonprotein ligand.

Several spectroscopic techniques have shown the existence of three paramagnetic forms of the NiFe enzymes called Ni−A, Ni−B, and Ni−C.[6] The Ni−A form corresponds to the solved X-ray structure[2] and is activated very slowly in the presence of H_2, whereas the Ni−B form is rapidly activated under the same conditions.[7] The Ni−C form of the enzyme is active and is two electrons more reduced than the Ni−B one.[8] It is generally considered as a form where hydrogen species such as H^-, H^+, or H_2 interact with the metal cluster.[9] In addition, EPR-silent forms called Ni−SI, Ni−Su, and Ni−R have been identified, and the proton exchanges associated with the various redox transitions have been characterized.[8,10]

Other important characteristics of NiFe-hydrogenases can be considered well grounded; X-ray absorption spectroscopy (XAS) experiments have indicated that no significant electron density changes take place on the Ni atom when as much as three electrons are accepted by the active site.[11a,12] Moreover, EPR[13]

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and ENDOR[14] experiments have shown that the iron ion remains diamagnetic, likely in an Fe^{II} low-spin state, in all the enzyme forms. However, a general agreement concerning the mechanism of dihydrogen activation and the nature of the various intermediates has not yet been reached.[2,10,13,15] Assuming that the Ni−C form is an intermediate species, the presence in this form of photolyzable hydrogen species bound to the Ni ion or located in its vicinity[16] led Dole et al.[13] to propose a catalytic cycle model which takes into account all available experimental data. In this model, H_2 activation is considered to occur in the Ni−SI to Ni−C steps, according to the mechanism proposed by Marganian et al.[13b] in which the heterolytic cleavage of H_2 takes place after binding on the Ni site by involving the basic properties of its terminal thiolate ligands, which act as proton acceptors. In contrast, in a recent ab initio investigation, Pavlov et al. have proposed, by using a model of the active site of NiFe-hydrogenase,[17] that the Fe center plays a major role in the catalytic process. However, despite the high quality of the calculations, the model does not fully agree with the available experimental data, especially those concerning the magnetic properties of the various forms of the enzyme. Therefore, its relevance to the mechanism of H_2 activation by NiFe-hydrogenases remains unclear.

To shed some light on the intriguing properties of the dinuclear [Ni−Fe] cluster of hydrogenases, we have carried out quantum mechanics (QM) calculations on Ni−Fe complexes modeling the active site of the enzyme, which were derived from the intermediate species involved in the catalytic cycle proposed by Dole et al.[13] The aim of these calculations was also to verify whether these active site models are energy-minima structures and whether their electronic properties are consistent with the available experimental data. In addition, we have also carried out calculations on models of the Ni−A form of the enzyme to compare the geometry obtained by QM calculations to that given by the X-ray crystal structure.

Methods

To perform the calculations using a high level of theory, the protein backbone was neglected and the four cysteine ligands of the protein were replaced by SH groups, following the work of Pavlov et al.[17] (Figure 1). The protein is, of course, expected to play a role in modulating the properties of the bimetallic cluster. However, as a first and necessary step toward a complete understanding of the enzymatic system, it is crucial to investigate the structural and electronic properties of the isolated [Ni−Fe] cluster. In the following, the model complexes used in this study are termed according to the nomenclature of the corresponding enzyme states proposed by Dole et al.[13] (Figure 1). The protonation state of these complexes was chosen according to the results of titration experiments.[10,15a] Besides, as observed also by Pavlov et al.,[17] only the terminal thiol ligands can be protonated without leading to unrealistic changes in the coordination sphere of the metal ions. Actually, to better study the localization of the electron added in the reduction of **B** and **C** to **SI** and **R**, respectively, no extra protons were added to the latter complexes (Figure 1). Concerning the investigation of the Ni−A state, two sets of calculations have been carried out. In the first model ($\mathbf{A_{XR}}$), all distances and angles have been kept fixed and equal to the corresponding X-ray values, while in the second model (**A**) the geometry was fully optimized with no external constraints. CH_3S and HS groups have been used to mimic the cysteine ligands in $\mathbf{A_{XR}}$ and **A**, respectively. It should be noted that the negative total charges of the complexes investigated are not unrealistic since they are similar to those carried by iron−sulfur clusters in proteins.[18]

The calculations were carried out with the Gaussian94 set of programs.[19] All complexes were subjected to a full geometry optimization without any symmetry constraints. The atomic basis set on first-row atoms was of double-ζ quality (D95[20]). Ne cores of S, Fe, and Ni atoms were replaced by Los Alamos ECP operators,[21] and the associated double-ζ basis functions were adopted.[21] The SCF calculations were carried out according to the density functional theory, using the exchange and correlation functionals proposed by Becke and Lee, Yang, and Parr, respectively (BLYP[22]).

Results and Discussion

The geometrical parameters obtained for the **A**, **B**, **SI, C**, and **R** complexes are reported in Table 1, where they are compared to the X-ray data for the *D. gigas* enzyme.[2] The differences observed in corresponding bond distances reflect the asymmetry of the complexes (see Figure 2) and indicate that the local environment plays a role, affecting differently similar bonds. However, the general structure of the dinuclear core is very similar in the **A** and $\mathbf{A_{XR}}$ complexes, as deduced by angles (Table 2) and torsion values (data not shown), indicating that it is not drastically affected by the protein moiety. In addition, the computed Fe−CO, Fe−CN, and Ni−Fe distances are very close to the experimental values. Our results show also that the Ni−Fe distance should strongly depend on the nature of the bridging ligands in the various enzyme forms. Such variations in the Ni−Fe distance have been effectively observed by EXAFS for several enzymes.[11] The differences in metal−sulfur bond distances between the $\mathbf{A_{XR}}$ and **A** complexes are likely due to the modeling of cysteinate ligands by simple SH groups and to steric effects of the protein backbone, here neglected. The influence of these differences on the electronic properties of the cluster is discussed below.

Apart from variations arising from changes of the bridging ligands, the structure of the Fe center is very similar in all complexes, whereas a modification of the geometry of the Ni center from a distorted square planar geometry (**B**, **SI**) to a distorted trigonal bipyramidal geometry (**C**, **R**) is clearly observed (Figure 2). However, due to steric constraints such a rearrangement may not necessarily take place in the protein. Most importantly, the optimized structures of the **C** and **R** complexes show that a hydrogen atom can effectively bridge the two metal centers, which leads to a shortening of the Ni−Fe distance of about 0.2 Å compared to $\mathbf{A_{XR}}$ and **A** and of about

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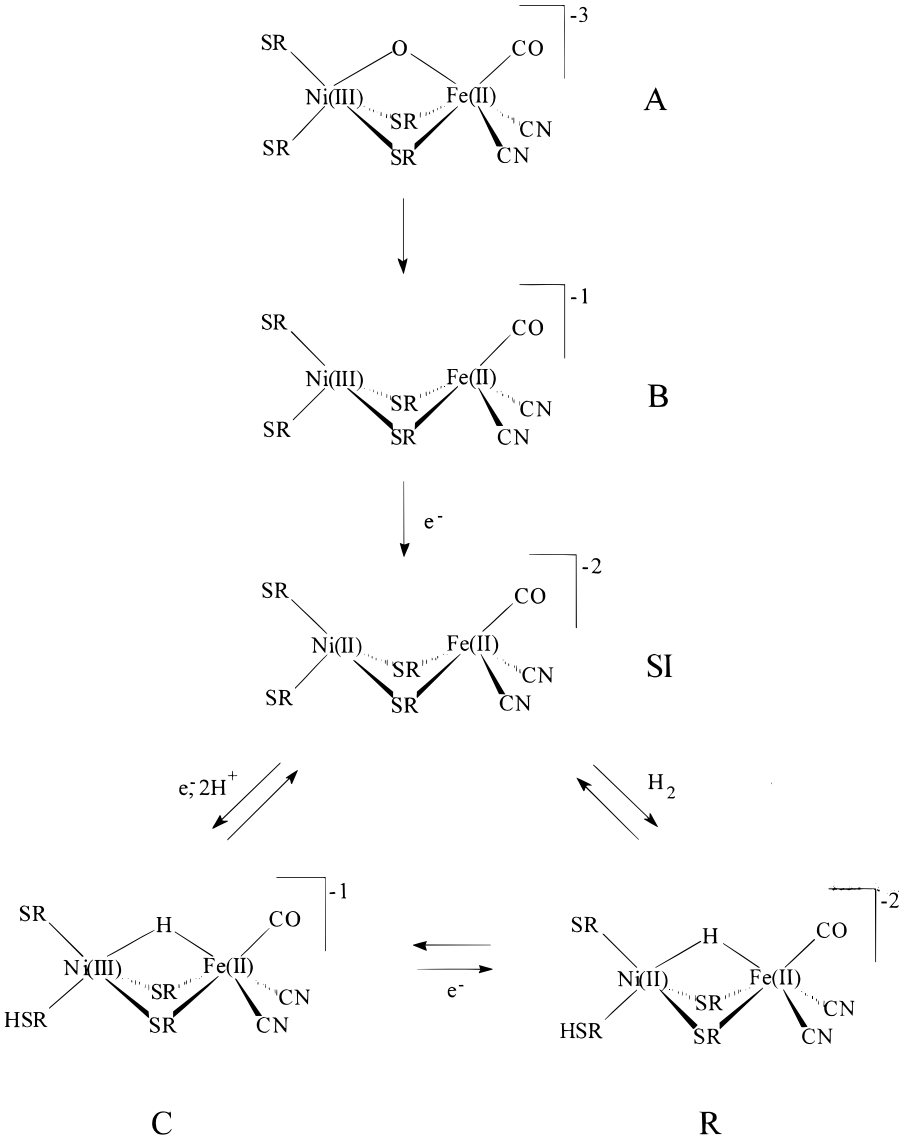

Figure 1. Intermediates in the activation of dihydrogen by NiFe-hydrogenases proposed by Dole at al.[13] To better follow the localization of the electron in the reduction of **B** and **C** to **SI** and **R**, respectively, no extra proton has been added to the latter complexes. In model complexes, R is a hydrogen atom in **A**, **B**, **SI**, **C**, and **R** and a methyl group in $\mathbf{A_{XR}}$.

Table 1. Computed Interatomic Distances in the Complexes **A**, **B**, **SI**, **C**, and **R**[a]

	$\mathbf{A_{XR}}$[b]	**A**[b]	**B**	**SI**	**C**	**R**
Ni−S_b	2.6	2.465	2.375	2.378	2.480	2.583
Ni−S_b	2.6	2.631	2.375	2.373	2.473	2.457
Ni−S_t	2.2	2.479	2.278	2.326	2.298	2.393
Ni−S_t	2.3	2.436	2.277	2.328	2.472[c]	2.334[c]
Ni−Fe	2.9	2.937	3.145	3.039	2.667	2.665
Fe−S_b	2.2	2.642	2.432	2.423	2.423	2.468
Fe−S_b	2.2	2.609	2.426	2.432	2.432	2.486
Fe−CO	1.7	1.763	1.717	1.708	1.734	1.722
Fe−CN(1)	1.9	1.913	1.888	1.904	1.900	1.919
Fe−CN(2)	1.9	1.915	1.889	1.905	1.902	1.911
Ni−H					1.552	1.521
Fe−H					1.850	1.930
Ni−O	1.7	1.857				
Fe−O	2.1	1.913				

[a] S_t and S_b are terminal and bridging sulfur ligands, respectively. [b] Complexes $\mathbf{A_{XR}}$ and **A** contain CH_3S and HS as ligands of the metal atoms, respectively (see Figure 1). [c] This atom is bound to two hydrogens in **C** and **R**.

0.4 Å compared to the unbridged **B** and **SI** complexes. Note that the Ni−Fe distances obtained in **C** and **R** compare well with the values deduced from EXAFS experiments carried out on the corresponding enzyme forms.[11a] Moreover, a recent X-ray crystallographic study performed on a strongly reduced state of the *D. gigas* hydrogenase has revealed a 0.3 Å shortening of the Ni−Fe distance by comparison with the Ni−A state.[11b] The good agreement between our results and these data strongly supports the existence of a bridging hydrogen atom in the Ni−C and Ni−R states of the enzyme. Actually, structures characterized by hydrogen bridges are quite common in inorganic compounds,[23] with metal−hydrogen−metal angle values falling in the range 78−124°[24] to be compared with values of 101−103° calculated for the **C** and **R** model complexes. Interestingly, **C** and **R** are among the few cases of "semibridged" hydrides,[23] with a Ni−H bond distance much shorter than the Fe−H one. It can be noted that the two bond distances between the Ni atom and the bridging sulfur atoms are rather different in **A** and **R**, due to a very weak interaction of one bridging S ligand with one hydrogen atom of the terminal SH or SH_2 ligand.

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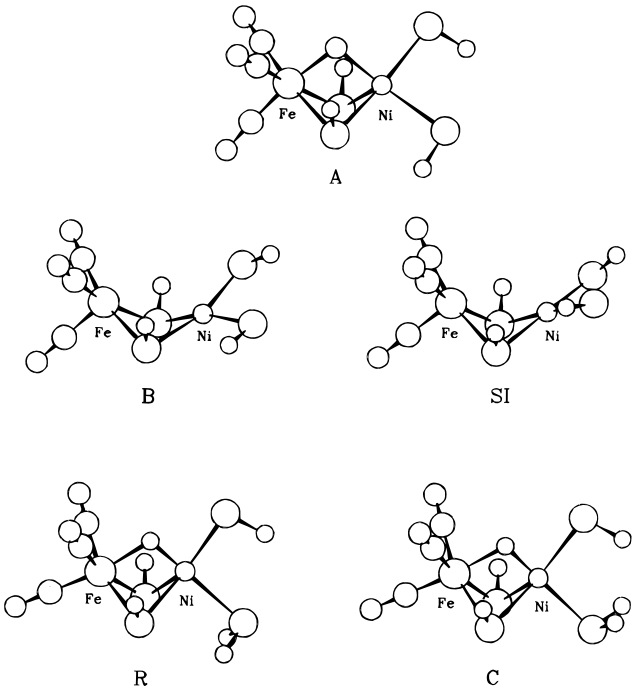

Figure 2. Structures of the complexes **A**, **B**, **SI**, **C**, and **R** obtained from DFT full geometry optimization.

Table 2. Computed Valence Angles in the Complexes **A**, **B**, **SI**, **C**, and **R**[a]

	$\mathbf{A_{XR}}$[b]	**A**[b]	B	SI	C	R
S_t−Ni−S_t	89	88.76	92.00	90.56	98.72	96.62
S_b−Ni−S_b	85	85.24	82.59	81.91	80.89	80.75
S_b−Fe−CO	91	91.64	99.00	92.47	105.70	102.86
S_b−Fe−CN(1)	171	172.41	166.34	160.41	163.39	169.59
CO−Fe−CN(1)	83	92.42	94.51	94.05	90.66	92.54
CO−Fe−CN(2)	94	93.17	92.24	92.24	90.15	91.31
CN(1)−Fe−CN(2)	91	92.57	95.16	94.29	93.00	92.62
Ni−S_b−Fe	74	70.67	81.83	78.84	65.75	65.52
Ni−S_b−Fe	74	62.70	81.71	79.05	65.99	61.93
Ni−H−Fe					102.89	101.06
Ni−O−Fe	97	102.35				

[a] S_t and S_b are terminal and bridging sulfur ligands, respectively. [b] Complexes $\mathbf{A_{XR}}$ and **A** contain CH_3S and HS as ligands of the metal atoms, respectively (see Figure 1).

One of the most debated questions concerning the various forms of NiFe-hydrogenases is the formal oxidation state of the two metal ions.[1] To investigate this point, the natural population analysis (NPA[25]) has been carried out on the electron densities associated with the complexes. NPA has been shown to produce more reliable and less basis set dependent results when compared to the Mulliken analysis.[25] The atomic partial charges for the various investigated complexes are reported in Table 3. The values associated with the metal ions and the ligands differ largely from those corresponding to the formal oxidation states, which underlines the covalent character of the metal−ligand bonds and shows that a discussion in terms of formal charges is inappropriate for this metal cluster. In particular, the Ni atom is characterized by very similar electron density values in all the complexes, and upon reduction from **B** to **R**, the partial charge change does not exceed 0.16 electron charge, which could explain the very small Ni K-edge shift observed by XAS upon reduction of the enzyme.[11a,12] Likewise, in all complexes, the Fe atom is characterized by very similar

Table 3. NPA Net Atomic Charges for the Complexes $\mathbf{A_{XR}}$, **A**, **B**, **SI**, **C**, and **R**[a]

	$\mathbf{A_{XR}}$[b]	**A**[b]	**B**	**SI**	**C**	**R**
Ni	0.65	0.84	0.62	0.51	0.57	0.46
Fe	−0.24	0.04	0.05	−0.01	−0.16	−0.13
S_b	−0.21	−0.54	−0.38	−0.43	−0.45	−0.58
S_b	−0.18	−0.58	−0.37	−0.43	−0.44	−0.67
S_t	−0.35	−0.77	−0.46	−0.62	−0.49	−0.51
S_t	−0.30	−0.67	−0.42	−0.62	−0.21[c]	−0.18[c]
CN(1)	−0.55	−0.51	−0.40	−0.48	−0.40	−0.49
CN(2)	−0.54	−0.49	−0.40	−0.48	−0.40	−0.47
CO	−0.02	−0.04	0.16	0.05	0.17	0.06
H					−0.01	−0.06
O	−0.69	−0.70				

[a] Natural population analysis (NPA, see ref 25). S_t and S_b are terminal and bridging sulfur ligands, respectively. [b] Complexes $\mathbf{A_{XR}}$ and **A** contain CH_3S and HS as ligands of the metal atoms, respectively (see Figure 1). [c] This atom is bound to two hydrogen atoms in **C** and **R**.

electron density values, and the electron density on CN and CO groups varies little. However, it is worth noting that the redox transitions from **B** to **SI** and from **C** to **R** are both accompanied by a small increase of the electron density on these diatomic ligands (Table 3), in agreement with the lowering of the stretching frequencies observed by IR spectroscopy in the enzyme.[3,4,10b] In fact, in these transitions, the extra electron is mainly accepted by the sulfur ligands, as already observed in the case of iron−sulfur clusters.[18,26] Thus, the electron density on the terminal sulfur increases significantly upon reduction of **B** to **SI**, whereas in the reduction of **C** to **R** the electron density change is mainly observed on the bridging sulfur (Table 3). It must also be pointed out that the partial charges observed for $\mathbf{A_{XR}}$ and **A** are very similar, indicating that the differences in bond lengths observed between the two complexes do not affect significantly their electronic properties.

Paramagnetic intermediates in the reversible activation of H_2 by NiFe-hydrogenases have been extensively characterized by EPR spectroscopy.[6,15a] However, the origin of their magnetism has been subject to debate since the $S = {}^1/_2$ ground-states observed by EPR could arise either from the coupling between two paramagnetic metal ions[27] or from the Ni center alone.[13] Moreover, the formation of sulfur radical species has been invoked to explain some experimental data.[12] To address some of these points, the spin density distribution was calculated by NPA for the low-spin paramagnetic complexes $\mathbf{A_{XR}}$, **A**, **B**, and **C,** and the results are reported in Table 4. We first observe that the spin density is always mainly localized on the Ni ion, in agreement with EPR experiments performed on ^{61}Ni-enriched hydrogenases.[6] In contrast, a negligible spin density is found on the Fe ion in the $\mathbf{A_{XR}}$, **A**, and **C** complexes, which is consistent with the results of ^{57}Fe ENDOR spectroscopy experiments.[14] A significant spin density is carried by the μ-oxo atom in the $\mathbf{A_{XR}}$ and **A** complexes, which explains the ^{17}O hyperfine coupling observed in the oxidized forms of the enzyme.[28] It is worth noting that the spin density distribution is not greatly affected by the small structural differences observed between $\mathbf{A_{XR}}$ and **A**. According to our calculations, an important fraction of the spin density is localized on the sulfur ligands in all complexes. Thus, in the **C** complex, the sizable spin density found on one of the terminal sulfur ligands may explain the ^{77}Se hyperfine coupling observed by EPR in NiFeSe hydroge-

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Table 4. NPA Spin Densities in $\mathbf{A_{XR}}$, **A**, **B**, and **C**[a]

	$\mathbf{A_{XR}}$[b]	**A**[b]	**B**	**C**
Ni	0.392	0.576	0.428	0.527
Fe	0.001	0.004	0.186	0.083
S_b	−0.002	0.047	0.006	0.128
S_b	0.166	0.165	−0.003	0.139
S_t	0.032	−0.015	0.172	0.192
S_t	0.000	0.016	0.217	0.001[c]
H				−0.032[d]
O	0.414[e]	0.211[e]		
$\langle S^2 \rangle$[f]	0.750	0.753	0.750	0.750

[a] Natural population analysis (NPA, see ref 25). S_t and S_b are terminal and bridging sulfur ligands, respectively. [b] Complexes $\mathbf{A_{XR}}$ and **A** contain CH_3S and HS as ligands of the metal atoms, respectively (see Figure 1). [c] This atom is bound to two hydrogens in **C**. [d] Hydrogen atom involved in the Ni−H−Fe bridge. [e] Oxygen atom involved in the Ni−O−Fe bridge. [f] Expectation value of the squared spin operator. The values are very close to 0.75, which corresponds to a pure doublet state.

nases in which a terminal cysteine ligand is replaced by a selenocysteine.[29] Interestingly, in this complex, the bridging H atom which is directly bound to the Ni ion carries almost no spin density, which accounts for the smallness of the proton hyperfine coupling measured in ENDOR experiments.[30]

The small spin density carried by the Fe ion in the **B** complex does not appear to be fully consistent with the lack of ^{57}Fe hyperfine coupling observed in the Ni−B form of the *D. gigas* enzyme.[14] This could result either from the influence of the protein environment on the magnetic properties of the Ni−B state or from a slight difference in the coordination of the metal center between the Ni−B form and the **B** model. In this respect, it should be noted that an oxygenic ligand was proposed to be present in this form on the basis of the effect of ^{17}O enrichment on the Ni−B EPR spectrum.[28]

Conclusions

The present QM investigation shows that, with the possible exception of the **B** complex, the structural and electronic properties of the active site models proposed by Dole et al.[13b] for the various forms of Ni−Fe hydrogenases are fully consistent with the available experimental data. This study confirms that, in all the enzyme forms that have been identified spectroscopically, the Fe ion remains in the same valence state. It shows also that the redox chemistry between these forms is not centered on only one of the metal ions but involves the whole cluster, especially the sulfur ligands. Further refinements using an improved description of the cysteine ligands are in progress to enable a more quantitative analysis of the experimental data and a better understanding of the Ni−B structure.

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