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High-Spin Ni(II), a Surprisingly Good Structural Model for [NiFe] Hydrogenase

Hua-Jun Fan and Michael B. Hall*

Department of Chemistry, Texas A&M University, TAMU 3255, College Station, Texas 77843-3255

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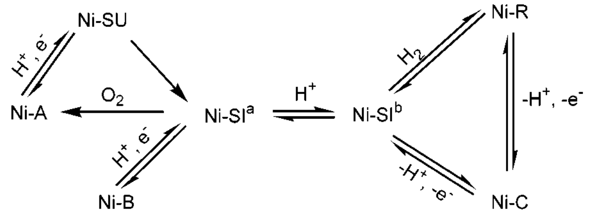
Density functional calculations on high-spin (HS) Ni(II) models for the active site of the [NiFe] hydrogenases predict a ligand arrangement about Ni that is in better agreement with the crystal structures than previous predictions for low-spin (LS) Ni(II) models.

The most well-studied of these enzymes, which catalyze the reversible reaction $H_2 \leftrightarrow 2H^+ + 2e^-$, are the [NiFe] hydrogenases.[1] Extensive experimental work[2] suggests the cycle shown in Figure 1. Of the seven clearly identified forms, Ni-A, -B, and -C are EPR active, while Ni-SU, Ni-SI(a,b), and Ni-R appear to be silent. Crystal structures of *Desulfovibrio gigas*, *Desulfovibrio vulgaris*, and *Desulfovibrio fructosovorans* (see Figure 2) show five- or six-coordinated Fe and four- or five-coordinated Ni, where the coordination number depends on the crystallized form.[3] In agreement with experimental results, theoretical calculations confirm that the Fe is LS Fe(II) for all forms, as expected for its strong-field CN^- and CO ligands.[4] However, the Ni oxidation state is not fixed, and both Ni(II) (Ni-SU, Ni-SI, Ni-R) and Ni(III) (Ni-A, Ni-B, Ni-C) are among the observed oxidation states, while Ni(I) may also be involved. All previous theoretical studies have assumed a LS state for the Ni(II) forms, which results in nearly planar Ni geometries.[4,5b] Although these geometries are in contrast to the crystal structures, where a distorted tetrahedral arrangement of S(Cys) is observed,[3] the discrepancy has been ignored because many of the crystal structures are five-coordinate about Ni and Ni(III) forms. However, the H-bonds to the cyanides and the protein backbone would need to accommodate a large rearrangement from the nearly tetrahedral Ni Cys arrangement observed in the crystal structure to the square planar Ni coordination previously predicted for the LS Ni(II) forms.

Although previous interpretation of the UV−visible MCD,[6] parallel mode EPR,[4i] and saturation magnetization experiments[7] suggested LS Ni(II), recent L-edge XAS experiments strongly suggest the existence of HS Ni(II) at the active site.[8] Support for the importance of the "tetrahedral" arrangement of S about Ni comes not only from the crystal structure but also from prediction of the cleavage reactions. Theoretical calculations predict that the H_2 cleavage has lower barriers and better overall energetics if the system is first oxidized to Ni(III).[9] The Ni(III) models for the dihydrogen precursor, transition state, and product for the heterolytic cleavage reaction have twist angles (between the NiS_2 bridge plane and the NiS_2 terminal plane) of 88°, 90°, and 78°.[9] These angles are close to the 88° angle found for the crystal structure of the oxidized form (a mixture of Ni-A and Ni-B forms) obtained from *D. gigas* and the 72°, 68°, and 73° angles found for *D. fructosovorans* and oxidized and reduced forms from *D. vulgaris*, respectively.



EPR signals: Ni-A, Ni-B, and Ni-C
EPR silent: Ni-SU, Ni-SI, and Ni-R

Figure 1. Proposed cycle for [NiFe] hydrogenase.

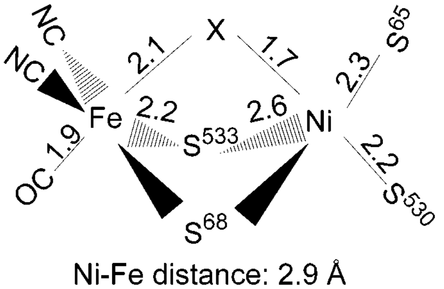


Figure 2. Crystal structure obtained from *D. gigas* hydrogenase. The sulfurs are cystein ligands; the "third" bridge (X) has been modeled as an O, OH, or OH_2 group for the oxidized form of *D. gigas* and as S for the oxidized form of *D. vulgaris*. Reduced forms have no heavy atom in this site.

In this Communication, we report the first fully optimized geometries (DFT)[10−13] on HS Ni(II) models for the Ni-SIa and Ni-R forms, $[(CO)(CN)_2Fe(\mu\text{-}SMe)_2Ni(SMe)_2]^{2-}$ and $[(CO)(CN)_2Fe(\eta^2\text{-}H_2)(\mu\text{-}SMe)_2Ni(SHMe)(SMe)]^-$, respectively. Previous experimental and theoretical work suggests that both Ni-SI and Ni-R forms are Ni(II) species, and that the Ni-SIb has one terminal cysteine protonated, while Ni-SIa has none. The Ni-R form is proposed to have dihydrogen bound to the active site. Although other models, such as ones with a bridging H between Ni and Fe, and with a terminal hydride on Ni, have been proposed for the Ni-R form,[14] comparing LS and HS Ni(II) models for both Ni-SIa and Ni-R will show that the general structure about the Ni(II) does not depend on the form being modeled.

The optimized geometries of the LS ($S = 0$) and HS ($S = 1$) models of both Ni-SIa and Ni-R forms are shown in Figure 3. The optimized geometries of the LS Ni(II) models for Ni-SIa and Ni-R are similar to those reported previously in a smaller basis set and have twist angles of 16.6° and 3.7°, respectively. On the other hand, the HS Ni(II) models have predicted twist angles of 87.4° and 87.9° for Ni-SIa and Ni-R model, respectively. Obviously, the latter angles are in much better agreement with those observed for the crystal structures. The HS geometries show somewhat longer Ni−S bond lengths. Although these distance may be somewhat too long because of a still relatively small basis set, the most recent EXAFS experiments also suggest longer Ni−S distances than those measured previously.[15] The longer Ni−S distances for Ni(II) are consistent with a HS model. These models also show slightly longer Ni−Fe distances than those found in the crystal structures, which have a third bridging atom. Little energetic difference is associated with shortening the Ni−Fe distance in the models with only two bridging atoms. Reoptimizing the geometry with the Ni−Fe distance fixed at 2.9 Å produces geometries similar to those reported above.[16]

The calculated energy differences show that the fully optimized geometries of HS Ni(II) models (**2** for Ni−SIa and **4** for Ni−R)

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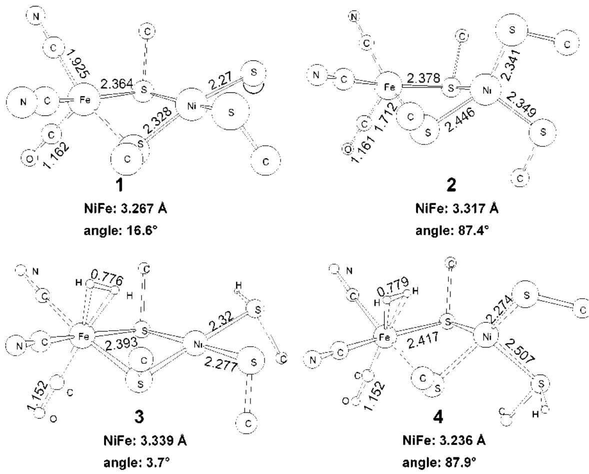

Figure 3. DFT model geometries (Å) for Ni-SIa, LS (1), HS (2), and Ni-R, LS (3), HS (4). The H's on methyl are not shown.

are more stable than the fully optimized geometries for the LS Ni(II) models (**1** for Ni−SIa and **3** for Ni−R) by 3.02 and 0.04 kcal/mol, respectively.[16] Although these differences appear small, the protein would have to be very flexible to accommodate both the pseudo-tetrahedral cysteine−Ni structures observed and the pseudo-square-planar structure predicted for LS Ni(II). If the protein resists the transformation from "tetrahedral" to "square planar", then the energy difference favoring HS Ni(II) could be even larger. For example, when the energy of the LS Ni(II) is determined at a geometry appropriate for the HS state, the LS state is ∼20 kcal/mol less stable.

Because both models, one for Ni-SIa and one for Ni-R, show such similar behavior, we are confident that these predictions are not being influenced by the models and that similar situations must exist in the active site of the enzyme. Furthermore, the calculated C−O distances appear to be independent of the Ni(II) spin state. Thus, the previous predictions for the active site species,[4a,b,5b] which used a correlation between the calculated C−O distance and the observed CO stretching frequency to establish oxidation states and proton counts at the active site, should remain valid. That is, HS Ni(II) can be accommodated easily into the previous scheme, as it is mainly a geometric change at Ni. Further support for HS Ni(II) comes from models for the CO inhibition studies,[17] where it is shown that HS Ni(II) models produce better correlation between predicted and observed CO stretching frequencies than LS Ni(II) models.

In conclusion, the freely optimized HS Ni(II) models for Ni-SIa and Ni-R show much stronger geometric resemblance to the X-ray crystal structure than the LS ones. With the crystal structures' geometry, the HS form is ∼20 kcal/mol lower in energy than the LS one. So, we conclude that HS Ni(II) is likely to be involved in these enzymes.

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Supporting Information Available: Detailed basis sets used in the calculations and the optimized geometry coordinates of species LS and HS of both Ni-SIa and Ni-R forms (PDF). This material is available free of charge via the Internet at http://pubs.acs.org/.

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