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Relativistic DFT Calculations of the Paramagnetic Intermediates of [NiFe] Hydrogenase. Implications for the Enzymatic Mechanism

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Hydrogenases are enzymes that catalyze the reversible heterolytic dissociation of molecular hydrogen $H_2 \rightleftarrows H^+ + H^-$. The largest class of hydrogenases contains a NiFe center that is believed to be the catalytic site for hydrogen activation.[1] Recent insight into the structure of the active site has come from X-ray structure analyses of single crystals of the [NiFe] hydrogenases from *Desulfovibrio* (*D.*) *gigas*[2] and *D. vulgaris* Miyazaki F.[3]

The active site (Figure 1) comprises a heterobimetallic cluster of Ni and Fe atoms. The bridging ligand X was proposed to be an oxygen or sulfur species in the oxidized states of *D. gigas* and *D. vulgaris*, respectively; X was found to be absent in the crystal structure of the reduced state of two enzymes.[4] Three nonprotein diatomics (2 CN and 1 CO) ligate the Fe atom.[5]

The "as-isolated" oxidized state of the [NiFe] hydrogenase is a mixture of two paramagnetic forms (Ni−A and Ni−B) with slightly different *g*-values.[1] Ni−B (or "ready") is reduced within minutes under an H_2 atmosphere while Ni−A (or "unready") requires incubation for several hours. An EPR-silent state (Ni−Si) is passed before a third paramagnetic state (Ni−C) and the fully reduced state (Ni−R) is obtained. Ni−C is believed to be an intermediate in the catalytic cycle. Upon illumination, the Ni−C state is converted into a fourth paramagnetic state (Ni−L). Carbon monoxide is an inhibitor of the enzyme yielding a paramagnetic CO-bound state (Ni−CO). All paramagnetic states are $S = {}^1/_2$. Previous quantum mechanical studies have addressed the question of H_2 activation by [NiFe] hydrogenases[6] and were mainly used to calculate IR transitions.[7] Here, we present the first relativistic[8] description and calculation of magnetic resonance parameters (*g*-tensors) of a transition metal containing enzyme[9] and show that these values can be correlated with structural parameters. This approach allows us to propose a reaction mechanism for the [NiFe] hydrogenases.

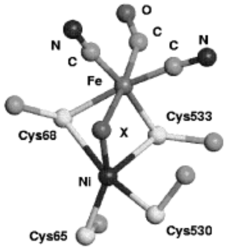

Figure 1. Active site of [NiFe] hydrogenase from *D. gigas* (predominantely in the Ni−A oxidation state)[2], Cys = cysteine.

Ni−B/Ni−A: The *g*-tensor magnitudes and orientations of the oxidized states were determined from EPR investigations of single crystals.[10] From the similarities of the *g*-values of Ni−A (2.32, 2.24, 2.01) and Ni−B (2.33, 2.16, 2.01), a drastic change in the electronic structure of the active site in the Ni−A state compared to Ni−B is unlikely. The *g*-tensor orientation was found to be very similar for Ni−B and Ni−A.[10] In the calculations, first the possibility of a sulfur species[3] (i.e. S^{2-}, SH^-, or H_2S) as bridging ligand X (Figure 1) was considered but did not lead to satisfying results.[11] Next, an oxygenic species was considered as X.[12] Such a species was postulated to occupy the position of the bridging ligand in *D. gigas*[2b] and *A. vinosum*.[13] The *g*-tensor orientation and the principal values of Ni−B were confirmed by our calculations when a OH^- ligand occupies the position of the bridging ligand (Table 1).[14] The calculated Ni−Fe distance is 3.00 Å, in reasonable agreement with the value obtained from the X-ray analysis of *D. gigas* (2.9 Å). ZORA calculations with a deprotonated bridging ligand, i.e., a O^{2-} bridge, gave *g*-values of 2.36, 1.95, 1.84. It was investigated whether the unrealistic values below g_e result from the spin-restricted nature of the wave function.[15,16]

The consideration of spin-polarization drastically improved the description. Thus a μ-oxo bridge was assigned to Ni−A. This is supported by the absence of D_2O exchangeable protons in the active center of the Ni−A state[17] and the required prolonged exposure to H_2 to be activated compared to Ni−B (see below). Protonation of a terminal cysteine as proposed in ref 7b did not yield satisfying *g*-values.[18] The postulated protonation of the O^{2-} bridge in the Ni−B form would not be detectable in the X-ray structure due to the limited resolution of 2.5 Å.[2b]

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(9) Computational details: The ZORA formalism as implemented in the Amsterdam Density Functional (ADF) package was used. The calculations are single-point calculations at nonrelativistic geometry-optimized structures using the BP86 exchange-correlation functional. Cysteines were modeled as $CH_3{-}CH_2{-}S^-$ groups. No constraints were imposed on the structures. A double-ζ Slater-type basis set with polarization functions (basis II in ADF nomenclature) was used. A triple-ζ basis set is used for the 3d shells of the first transition metals. The following orbitals were frozen during geometry optimizations: C 1s, N 1s, O 1s, S up to 2p, Ni up to 2p, Fe up to 2p. The calculations of magnetic resonance parameters were performed in an all-electron basis.

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(11) After complete geometry optimizations the Ni−Fe distances are 3.15 Å for H_2S, 3.11 Å for SH^-, and 3.19 Å for S^{2-}, whereas the X-ray structures yield 2.55 Å for *D. vulgaris* Miyazaki F and 2.90 Å for *D. gigas*. The calculated *g*-values (g_x, g_y, g_z) are 2.19, 2.06, 2.01 for a H_2S, 2.19, 2.15, 1.99 for a SH^-, and 2.31, 2.07, 1.91 for an S^{2-} ligand. These are not in good agreement with experimental values.

(12) Comparison of structural data from X-ray analysis and geometry optimizations are available as Supporting Information.

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Table 1. Comparison of Experimental and ZORA Calculated *g*-Tensor Principal Values for [NiFe] Hydrogenase Paramagnetic States

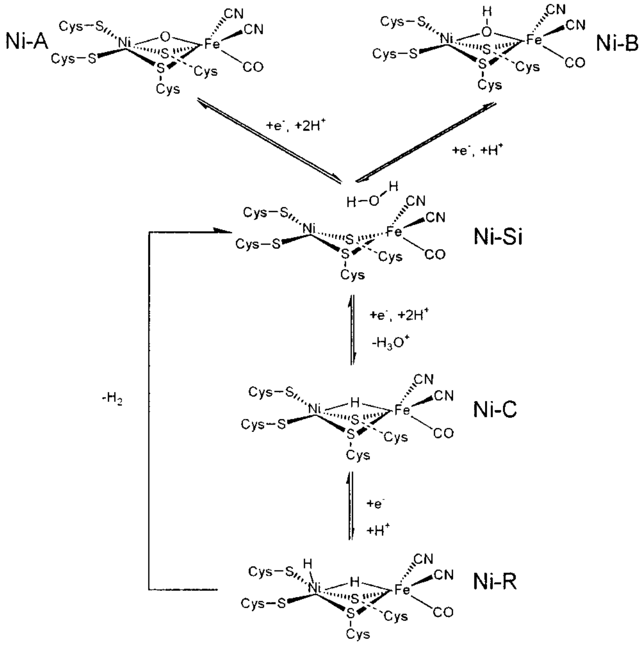
		g_x	g_y	g_z
Ni−B	exptl	2.33	2.16	2.01
	calcd	2.21	2.17	1.98
Ni−C	exptl	2.20	2.14	2.01
	calcd	2.19	2.10	2.00
Ni−L	exptl	2.29	2.12	2.05
	calcd	2.26	2.10	2.05
Ni−CO	exptl	2.12	2.07	2.02
	calcd	2.11	2.06	2.00

Ni−C: In the X-ray structure analysis of the reduced enzyme, the position of the bridging ligand is vacant.[4] Either the substrate or the dissociation products may, however, be bound to the active center that would not be detectable by X-ray crystallography. We found that only when a hydride ion occupies the position of the bridging ligand[19] and the Ni is in a formal Ni(III) oxidation state do the calculated *g*-tensor principal values agree well with those found experimentally (see Table 1). The assumption of a hydride bridge is in agreement with the observation of a large, D_2O-exchangeable ^1H hyperfine coupling in the Ni−C state.[20] Formally, Ni−C is two electrons more reduced than the oxidized Ni−A/Ni−B states. Recent EXAFS results, however, do not report a large shift in electron density at the Ni atom.[21] This is in agreement with our model that Ni−C contains Ni(III) which implies that a reduction in the ligand sphere takes place upon Ni−A/B → Ni−C conversion.

Ni-L: Upon illumination of the Ni−C state at cryogenic temperatures a new rhombic signal evolves (Ni−L) (see Table 1).[1] The reaction is reversible and tempering at 180 K fully recovers the Ni−C signal. In Ni−L photodissociation of a ligand has been proposed since the large ^1H-ENDOR coupling is lost after photolysis.[20] Removal of a proton from the bridging position leads to a formal Ni(I) state and good agreement with the experimental *g*-values was obtained (Table 1).[22]

Ni−CO: The hydrogenase is irreversibly inhibited by binding of exogenous CO. This is accompanied by a change of the *g*-values (see Table 1) and observation of a large isotropic ^{13}C hyperfine coupling of 85 MHz when ^{13}CO is used.[13] Recently, Happe et al. proposed that the Ni−L state binds CO and not Ni−C.[23] Calculations on a Ni(III) species with an axial CO bound to the Ni and a vacant bridging position lead to calculated values of 2.20, 2.03, 2.00 and may thus be ruled out. Only if one assumes that the Ni(I)−L cluster model binds CO in an axial position do the calculated *g*-tensor principal values agree well with experimental data (Table 1).[24] The isotropic ^{13}C hyperfine interaction of CO is also well reproduced (72 MHz).

Scheme 1. A Proposed Mechanism for the Catalytic Cycle of [NiFe] Hydrogenase



Reaction Mechanism: Based on the structures of the intermediates suggested here, a reaction model for the heterolytic splitting of molecular hydrogen can be proposed (see Scheme 1): In the activation cycle, the bridging ligand of Ni−B (OH^-) is protonated and remains only loosely coordinated in the Ni−Si state as H_2O. This water may assist the heterolytic cleavage of molecular hydrogen by taking up a proton. The hydride then occupies the position of the bridging ligand in the Ni−C state and H_3O^+ is released. We further suggest that in the fully reduced, diamagnetic Ni−R state an additional hydrogen atom (or a proton upon one-electron reduction of Ni) is terminally bound to the Ni atom. Upon regeneration of the catalyst, H_2 may be released and the catalytically active Ni−Si state is recovered.

Experimentally observed magnetic resonance parameters have been correlated with the composition and geometry of the related paramagnetic intermediates in the catalytic cycle of an important enzyme resulting in the proposal of a reaction mechanism. Furthermore, light-induced changes and inhibition of the enzyme could be explained. The relativistic DFT approach of the ZORA Hamiltonian proved to be reliable to calculate *g*-tensors of a complex metalloenzyme, with the exception of the Ni−A state where spin-polarization effects are important.

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Supporting Information Available: Comparison of structural data from X-ray analysis and geometry optimizations (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) Spin-unrestricted quasi-relativistic (QR) calculations using the Pauli-Hamiltonian but otherwise the same basis set, exchange-correlation functional, and integration scheme yielded values of 2.187, 2.148, 2.028 for X = OH^- and 2.183, 2.159, 2.046 for X = O^{2-}. These values follow the experimental trends observed for Ni−B (OH^-) and Ni−A (O^{2-}), respectively. Spin-unrestricted QR calculations on Ni−C, Ni−L, and Ni−CO states gave *g*-values not very different from those of spin-restricted ZORA results. Only the results of Ni−A showed larger deviations; in the spin-restricted calculations of the Ni−A state the energy of the highest fully occupied molecular orbital was almost degenerate with that of the singly occupied molecular orbital. In such cases spin-polarization effects on the calculated *g*-values can be particularly large. When one assumes that the differences between the Ni−A and Ni−B forms from spin-polarized QR calculation can be transferred to the ZORA results for Ni−B, reasonable values for Ni-A are obtained (2.21, 2.19, 2.01).

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