Modeling the Active Sites in Metalloenzymes 5. The Heterolytic Bond Cleavage of H_2 in the [NiFe] Hydrogenase of *Desulfovibrio gigas* by a Nucleophilic Addition Mechanism

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Received July 10, 2001

The H_2 activation catalyzed by an Fe(II)−Ni(III) model of the [NiFe] hydrogenase of *Desulfovibrio gigas* has been investigated by density functional theory (DFT/B3LYP) calculations on the neutral and anionic active site complexes, $[(CO)(CN)_2Fe(\mu\text{-}SH)_2Ni(SH)(SH_2)]^0$ and $[(CO)(CN)_2Fe(\mu\text{-}SH)_2Ni(SH)_2]^-$. The results suggest that the reaction proceeds by a nucleophilic addition mechanism that cleaves the H−H bond heterolytically. The terminal cysteine residue Cys530 in the [NiFe] hydrogenase active site of the *D. gigas* enzyme plays a crucial role in the catalytic process by accepting the proton. The active site is constructed to provide access by this cysteine residue, and this role explains the change in activity observed when this cysteine is replaced by a selenocysteine. Furthermore, the optimized geometry of the transition state in the model bears a striking resemblance to the geometry of the active site as determined by X-ray crystallography.

Introduction

Hydrogenases are of great biotechnological interest because of their role in environmentally acceptable hydrogen production technology.[1−3] As the central feature of hydrogen activity in microorganisms, hydrogenases have the ability to catalyze the reversible splitting of dihydrogen ($H_2 \Leftrightarrow 2H^+ + 2e^-$). Generally, these organisms either oxidize molecular hydrogen to generate reducing power or produce hydrogen as a sink for excess electrons.[1,2] A series of recent experimental[4] and theoretical[5] studies have been concerned with the catalytic mechanism of this system and the structure of its intermediates.

Generally, hydrogen activation by transition metal complexes proceeds through either oxidative addition or σ-bond metathesis (which is usually described as homolytic and without strong charge polarization, unless R is a polar group) processes, as shown in Scheme 1.[6] Recently, a new type of hydrogen bonding, M−H···H−X (X = N, O) between a conventional hydrogen bond donor, as the weak acid component, and a metal hydride bond, as the weak base component, has been reported for both intra- and intermolecular interactions.[7] Deuteration experiments suggest that the H···H interaction is essential for rapid H/D exchange with D_2 gas. Morris and co-workers suggested that the H/D exchange takes place via initial intramolecular proton transfer from $N-H^+$ to $Ir-H^-$ to generate a η^2-H_2 complex which can then exchange readily with D_2 gas, as illustrated in Scheme 2.[7f] This exchange process clearly involves heterolytic bond cleavage of D_2/H_2.

More recently, Dedieu and co-workers found that the cleavage of H_2 by $[Rh(PH_3)_2(O_2CH)\cdots NH_3]$ to generate $[Rh(H)(PH_3)_2(HO(O)CH)\cdots NH_3]$ might occur not only by a σ-bond metathesis mechanism but also by a heterolytic bond cleavage mechanism.[8a,b] The calculated results at the MP2//MP2 level

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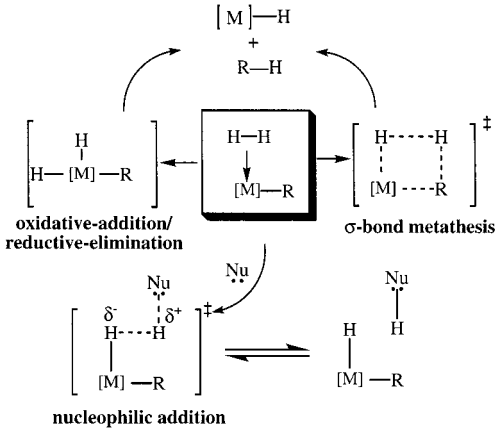
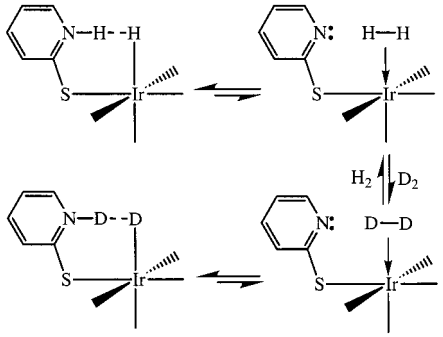
10.1021/ic0107274 CCC: $20.00 © 2001 American Chemical Society
Published on Web 10/17/2001

Scheme 1



Scheme 2



show that the latter pathway is exoenergetic by 13.6 kcal/mol with two quite low barriers of < 2 kcal/mol, while the former pathway is endoenergetic by 1 kcal/mol with a barrier of 11 kcal/mol. In their experimental and theoretical studies of the reduction of water to H_2 mediated by bis-alkyl tris(pyrazolyl)-borate Pd(II) complexes, Dedieu, Canty, and co-workers also investigated the reverse of heterolytic cleavage, where dihydrogen is generated from a system having a M−H···H−X "dihydrogen bond".[8a,c] The calculated results show that the energy barrier for this elimination depends on the nature of the M−H bond. Since these heterolytic cleavage processes of H_2 by M and X to form $M-H^{-}\cdots H^{+}-X$ are initially related to polarizing the H_2 bond, $H^{\delta-}-H^{\delta+}$, on the approach of nucleophilic substituent, X, nucleophilic addition is the common feature of these reactions (Scheme 1).[6]

Theory

Theoretical calculations in this work have been performed with the DFT method,[9] specifically with the Becke three parameter hybrid exchange functional[10] and the Lee−Yang−Parr correlation functional[11] (B3LYP). The optimized stationary points were characterized by frequency calculations.[12] The basis sets for iron and nickel are modified versions of the Hay and Wadt basis set with effective core potentials[13a] (ECP). The modifications to the double-ζ basis set were made by Couty and Hall[13b] and give a better representation of the 4p space. The resulting basis set is a (341/541/41) contraction for iron and nickel, where the 3s and 3p basis functions are left totally contracted but the 4s, 4p, and 3d are split. For carbon, nitrogen, oxygen, and sulfur, the ECPs and basis sets of Stevens, Basch, and Krauss[14] were used in double-ζ form with polarization functions. The Dunning−Huzinaga (31) double-ζ basis set was used for the hydrogen atoms.[15] Natural population analysis (NPA) and natural bond orbital analysis[16] (NBO) were performed at the B3LYP/6-31G** level.[17] In our computational models, we neglected the protein backbone and replaced cysteines 65, 68, 530, and 533 of the large subunit in the actual [NiFe] *D. gigas* hydrogenase by SH^{-}.[18] All DFT calculations were performed with the GAUSSIAN98[17] and NWChem programs.[19]

Results

In previous work[5c] on a wide variety of redox and protonated structures of the active-site model $[(CO)(CN)_2Fe(\mu\text{-}SMe)_2Ni(SMe)_2]^{n-}$, we found that dihydrogen activation may involve proton transfer to a terminal cysteine residue and is more exothermic on the Ni(III) species than on the corresponding Ni(II) or Ni(I) species. Here, B3LYP geometry optimizations were carried out for possible structures of the active site along a nucleophilic addition pathway for the Ni(III) species with a simpler model, where Me groups are replaced by H. This replacement causes only small differences in the optimized structures, but makes the S less basic and reaction barriers higher. These structures include the anionic H_2 associated species **1**, $[(CO)(CN)_2Fe(\eta^2\text{-}H_2)(\mu\text{-}SH)_2Ni(SH)_2]^{-}$, the neutral H_2 associated species **4**, $[(CO)(CN)_2Fe(\eta^2\text{-}H_2)(\mu\text{-}SH)_2Ni(SH)(SH_2)]^{0}$, their transition states, **2** and **5**, and their bridging hydride products, **3** and **6**, as illustrated in Figure 1. The calculated relative Gibbs free energies (ΔG) (see Figure 1) show that H_2

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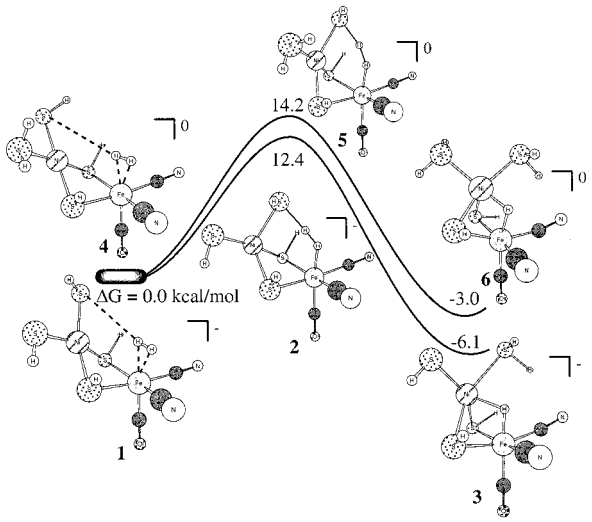

Figure 1. The B3LYP optimized geometries and calculated relative Gibbs free energies (ΔG) for the H_2 activation catalyzed by the active site along nucleophilic addition pathway for the neutral and anionic Ni(III) species.

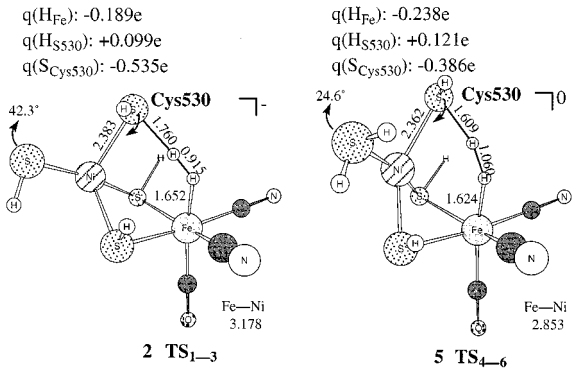

Figure 2. The important geometric parameters at the B3LYP/ECPDZ level, and the NPA parameters at the B3LYP/6-31G** level for transition states **2** and **5**.

activation by the anionic active site is exothermic by 6.1 kcal/mol with a barrier of 12.4 kcal/mol, whereas H_2 activation by the neutral active site is exothermic by 3.0 kcal/mol with a barrier of 14.2 kcal/mol.

The important geometric and NPA parameters for the transition states are shown in Figure 2. Note that in the transition states the torsion angles, 42.3° and 24.6°, of the $Ni(S_{terminal})_2$ plane relative to the $Ni(S_{bridge})_2$ plane clearly increase by about 20° with respect to those of **1** and **4**, respectively. Since the experimentally observed torsion angle of the [NiFe] hydrogenase of *D. gigas* is about 34°,[2,4a,b] the geometric similarity of the terminal cysteine position in the proposed transition state **2** and in the active site of the enzyme suggests that the protein backbone may assist in decreasing the reaction barrier for H_2 cleavage.

As the H_2 associated with Fe is attacked by the cysteine Cys530 residue, H−H is polarized such that the hydrogen atom bound to Fe acquires a negative charge and that bound to S_{Cys530} takes a positive charge. This heterolytic bond cleavage has structural and electronic features consistent with a nucleophilic addition process. In comparison to the neutral transition state **5**, the anionic system shows an earlier transition state (**2**) because the larger torsion angle leads to an increase in the basicity of S, which favors the nucleophilic addition process. Generally, perturbations that bring about the following two changes in the nucleophile will render the nucleophilic attack on H_2 more favorable: one is to increase the lone-pair electron density of the nucleophile and the other is to destabilize the lone-pair orbital. Recently, the EPR[20] and X-ray[21] experimental observations showed that the H_2/HD ratio in the proton−deuteron exchange reaction is more than doubled when the terminal cysteine Cys530 of the [NiFe] hydrogenase of *D. gigas* is replaced by a selenocysteine (SeCys) residue (as in the [NiFeSe] hydrogenase of *Desulfomicrobium baculatum*). This result is somewhat surprising because a selenol group is more acidic than a thiol (pK_a of SeCys is 5.2 whereas that of Cys is 8.0).[22] Since the [NiFe] and [NiFeSe] hydrogenases have similar structures and reactions, the higher nucleophilicity of the SeCys residue could be explained in terms of its higher-lying lone-pair orbital with respect to that of the Cys residue. Alternatively, if the ratio is related to how the electrophilic proton on the Cys or SeCys residue is donated to an acceptor in its vicinity, then the higher acidity of SeCys could easily explain these observations. In either case, the terminal residue, Cys530 in the *D. gigas* enzyme and SeCys487L in the *D. baculatum* enzyme, appears to play a crucial role in the catalytic process. The direct involvement of this terminal S(Se) ligand seems to preclude previously proposed mechanisms in which the bridging cysteine residue attacks H_2 followed by proton transfer to a terminal S ligand. Furthermore, this other mechanism involves large changes in the structure of the three nonprotein ligands and of the protein chain connected the bridging cysteine residue (Cys68 or Cys533) with respect to the experimentally observed enzyme structure. Additional calculations show that the barrier is higher if one uses the bridging S as the initial base.

Conclusions

By combining the experimental observations, our previous theoretical work, and the work described here, we have been able to make a reasonable conclusion about the reaction mechanism of the H−H activation step as catalyzed by the [NiFe] hydrogenase. The H_2 cleavage proceeds by a heterolytic cleavage along a nucleophilic addition pathway, in which the electronic and steric properties of the terminal Cys or SeCys residues play a key role. Furthermore, the optimized TS structure for this simple unconstrained model complex bears a striking resemblance to the crystal structures of the active site. Future studies of the [NiFe] hydrogenases including solvent effects,[23] inactive states,[24] and high-spin Ni(II)[25] are underway.

Acknowledgment. This work was supported by the National Science Foundation (CHE9800184). This research was performed in part using the Molecular Science Computing Facility (MSCF) in the William R. Wiley Environmental Molecular Sciences Laboratory at the Pacific Northwest National Laboratory. The MSCF is funded by the Office of Biological and Environmental Research in the U.S. Department of Energy. PNNL is a multiprogram national laboratory operated for the U. S. Department of Energy by Battelle Memorial Institute under contract DE-AC06-76RLO 1830. S.Q.N. also acknowledges helpful discussion with HPCC group members about the NWChem code.

IC0107274

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