Hydrogenation without a Metal Catalyst: An ab Initio Study on the Mechanism of the Metal-Free Hydrogenase from *Methanobacterium thermoautotrophicum*

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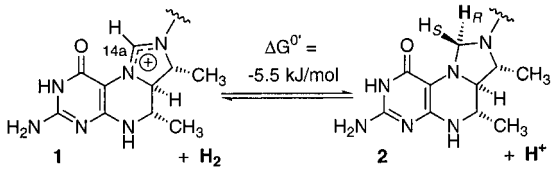
Hydrogenases are enzymes that catalyze reactions involving molecular hydrogen (H_2) either as substrate or as product. All hydrogenases investigated so far are metalloenzymes: they all contain iron−sulfur clusters, and most of them contain nickel.[1] Most likely, the activation of the H_2 molecule takes place at one of the redox-active transition metal ions. Recently, a new hydrogenase was found in the methanogenic archaeon *Methanobacterium thermoautotrophicum* which catalyzes the reaction of its substrate N^5,N^{10}-methenyl tetrahydromethanopterin (methenyl-H_4MPT, **1**, Scheme 1) with hydrogen *without* the aid of nickel or iron−sulfur clusters. In other words, H_2-forming methylene tetrahydromethanopterin dehydrogenase—as the enzyme is called—is a purely organic hydrogenation catalyst![2]

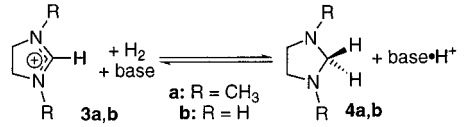
The existing body of data for this most intriguing catalytic process strongly suggested that the reaction shown in Scheme 1 is brought about by enzymatic activation of the substrate **1**.[3] Carbocations such as the *tert*-butyl cation are known to react with molecular hydrogen in a way that a formal hydride transfer occurs, giving rise to the saturated hydrocarbon (e.g. isobutane) and a proton.[4,5] Obviously, this is analogous to what happens in the enzymatic reaction. The question results how the stable formamidinium cation **1** is transformed into a "hot" carbocation. It was recently proposed that protonation of the amidinium nitrogen atoms in the enzyme's active site and/or an enzyme-induced distortion of the 5-membered ring from planarity to an envelope conformation may effect the necessary increase in reactivity[3] of **1**.

In fact, a recent *ab initio* calculation confirmed the expected increase in reactivity upon folding.[6] Cioslowski and Boche calculated a reaction enthalpy of +138 kJ/mol in the model transformation of **3a** to **4a** (Scheme 2) when (monomeric) H_2O was employed as the proton acceptor.[6] However, the enzyme-catalyzed reduction of **1** with hydrogen is almost thermoneutral[2] ($\Delta G^{\circ\prime} = -5.5$ kJ/mol), as expected for a reversible reaction. This discrepancy shows that the calculation cited[6] did not provide a viable model for the overall enzymatic transformation. The most likely reason for this result is that an isolated water molecule was considered instead of bulk water which serves as the final proton acceptor in the enzymatic reaction (Scheme 1). We herein disclose the results of our own ab initio calculations on the reaction of formamidinium cations of the type **3** with hydrogen. We show that the reaction enthalpy is strongly dependent on whether a single water molecule, dimer, trimer, or tetramer is used. On the basis of these calculations, we propose that a primary amine, such as the ω-amino group of a lysine residue, most likely acts as the primary proton acceptor in the enzyme's active site.

Scheme 1



Scheme 2



Using the model transformation **3b** → **4b** (Scheme 2) and an isolated water molecule as the base, the computed[7] reaction enthalpy is +210 kJ/mol.[8] If one instead uses the hydrogen-bonded water dimer,[18,19] the reaction enthalpy drops to only +70 kJ/mol and becomes still lower for larger water clusters: +14 kJ/mol for the trimer[18,20] and −55 kJ/mol for the tetramer **5** (Figure 1).[18] Obviously, the reaction enthalpy is strongly dependent on the model acceptor employed, and the approximate thermoneutrality of the enzymatic reaction can easily be reproduced by using a proton acceptor (i.e., water aggregate) of suitable basicity.

To further show the effect of base strength on the reaction enthalpy, we performed analogous calculations using ammonia, methyl-, dimethyl-, and trimethylamine as "base" in Scheme 2.

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(7) All quantum chemical computations have been performed using the TURBOMOLE program package.[9,10] The modules for geometry optimization, second derivatives, and energy have been parallellized for the SGI Power Challenge.[11] The geometries of the systems of interest have been calculated by an RI-DFT approach,[12,13] with the Becke−Perdew functional[14,15] and split-valence basis sets.[16] For C and N, the basis sets have been augmented by one d polarization function (SV(P)). The geometry optimizations have been considered to be converged when the change in energy dropped below 1 μHartree (2.6 J/mol), and the norm of the Cartesian gradients became less than 1 mHartree/bohr (1.4 kJ/Å). Saddle points have been located by a 1D search along an appropriate reaction coordinate. The mathematical properties of the saddle points were checked by the calculation of the matrix of the second derivatives of the energy with respect to the nuclear coordinates by means of a finite difference procedure. Energies were obtained on MP2-level (with frozen core) using TZVP basis sets.[17]

(8) We assume that the difference in reaction enthalpy found by Cioslowski and Boche (+138 kJ/mol)[6] and us (+210 kJ/mol) is due to the different computational methods employed. Unfortunately, Cioslowski and Boche did not disclose computational details.

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(18) Our computed structure of the water dimer (trimer) is almost identical with the one given in ref 19 (20). Our computed structure of tetramer **5** is shown in Figure 1. The structures of the protonated water oligomers were derived from that of the protonated tetramer (**5**•H^+) in Figure 1 by successively removing the outer water molecules.

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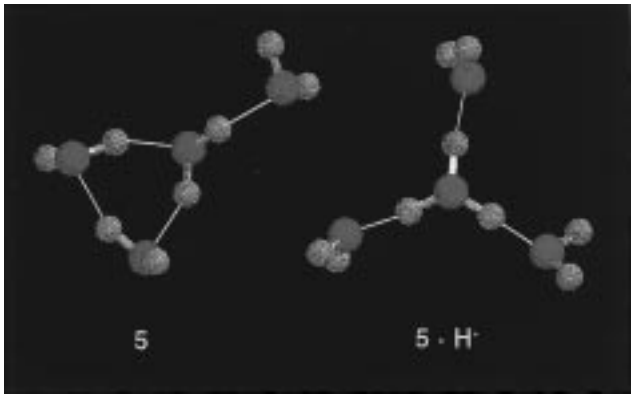
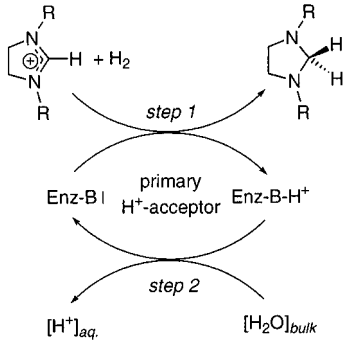


Figure 1. Computed Structure of the water tetramer **5** and the protonated water tetramer (**5**·H^+).

Table 1. Calculated Enthalpies of the Reaction Shown in Scheme 2 (**3b** and **4b**) and Measured Proton Affinities (pa)[21] of the Bases

base	ΔE [kJ/mol]	pa [kJ/mol]
NH_3	+36.6	+871.5
CH_3NH_2	−8.4	+918.8
$(CH_3)_2NH$	−38.5	+953.1
$(CH_3)_3N$	−57.1	+973.6

Scheme 3



The results are summarized in Table 1. Obviously, the reaction energy is strongly dependent on the strength of the bases and correlates linearly with their measured gas-phase proton affinities. The excellent correlation coefficient ($\rho^2 = 0.9994$) clearly demonstrates the high quality of the calculations. Of course, increasing the basicity of the primary proton acceptor in the enzyme's active site will increase the driving force for this initial proton transfer (step 1, Scheme 3). However, an initial exothermic proton transfer results in an extremely high activation barrier for the subsequent step (step 2, Scheme 3). Consequently, an (approximately) thermoneutral initial proton transfer appears much more favorable in view of the overall reaction.

Inspection of Table 1 reveals that a primary amine would make an ideal primary proton acceptor. In the enzyme, this functionality may well be provided by the ω-amine group of a lysine residue. To probe the kinetic feasibility of step 1 in Scheme 3, we located the transition state for the reaction of **3b** with hydrogen in the presence of methylamine as base, using high-level ab initio calculations. Interestingly, the reactants spontaneously preorganized to form a properly oriented van der Waals complex (**A**, Figure 2). The formation of this complex is exothermic by 16.7 kJ/mol, with respect to the separated reactants in vacuo.[22] Starting from this complex, the transition state (**B**, Figure 2) could easily be located, and it was found to lie only 82 kJ/mol above the van der Waals complex **A**. This transition state has only one imaginary vibration frequency (382*i* cm^{-1}), and the molecular motions are as expected, connecting the reactants and the products. The experimentally determined (Arrhenius plots) activation energies for the reverse reaction, i.e., the formation of hydrogen from **2** (Scheme 1) are in the range of 35−70 kJ/mol for the H_2-forming methylene tetrahydromethanopterin dehydrogenases from various organisms.[23] "Step 2" in Scheme 3 is not expected to be rate-determining. Taking into account that our model system **3b**/**4b** is a simplified version of the natural substrate and assuming that the enzyme may further stabilize the transition state (**B**, Figure 2), our calculated value for the activation barrier of 73.7 kJ/mol appears quite reasonable.

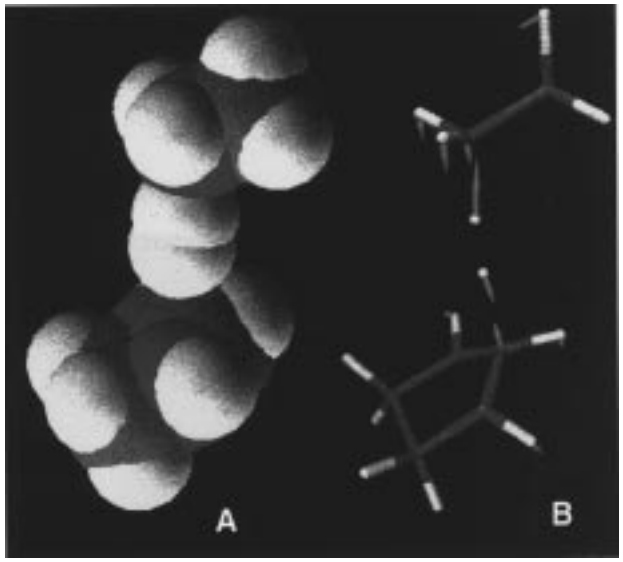


Figure 2. Ternary van der Waals complex **A** and transition state **B** found for the reaction of the amidinium cation **3b**, H_2, and methylamine. For transition state **B**, the normal vectors for the only vibration with an imaginary frequency are shown as red arrows.

In summary, we have reached the following conclusions concerning the thermodynamics and kinetics of the reaction catalyzed by H_2-forming methylene tetrahydromethanopterin dehydrogenase (Scheme 1): (a) The approximate thermoneutrality of the enzymatic transformation may well be reproduced by ab initio calculations if the higher basicity of bulk water is taken into account. (b) There is a low-energy pathway for the reduction of an amidinium cation with hydrogen in the presence of an amine base. Along this pathway, no initial distortion of the formamidinium cation from planarity is required. (c) On the basis of the calculated reaction enthalpy and the geometry of the transition state, we predict that in the enzyme's active site, there should be a basic nitrogen atom (most likely, the ω-amino group of a lysine residue) located on the *re* side of the reactive carbon atom (14a) of the substrate **1**, at a distance of ca. 460 ± 80 pm. We believe that the results presented here do not only shed light on the mechanism of a most intriguing enzymatic transformation but also are expected to pave the way for the construction of functional model systems.

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