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Catalytic Mechanism of the Metal-Free Hydrogenase from Methanogenic Archaea: Reversed Stereospecificity of the Catalytic and Noncatalytic Reaction**

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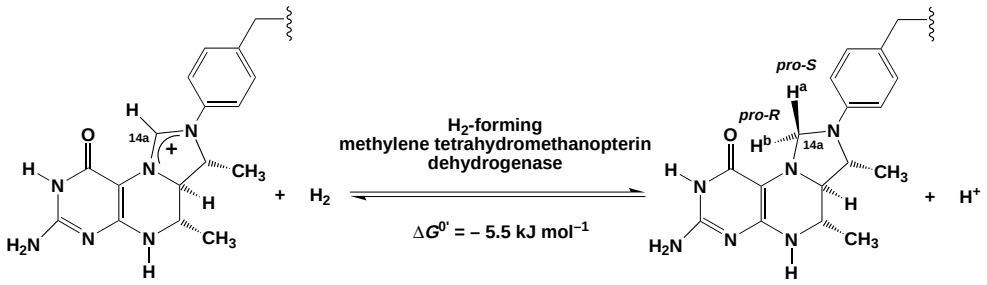
Hydrogenases are enzymes that catalyze reactions with molecular hydrogen (H_2) either as substrate or as product.[1] They usually contain a redox-active nickel/iron or iron center that binds and activates H_2. Typically hydrogenases also contain several iron–sulfur clusters that transfer electrons to an electron acceptor.[2–4] In addition, most methanogenic archaea express a metal-free hydrogenase which, therefore, can neither activate H_2 nor catalyze electron transport.[5] All available evidence indicates that this enzyme activates the hydrogen acceptor, which then directly reacts with H_2.[6]

The metal-free hydrogenase catalyzes the reversible reduction of N^5,N^{10}-methenyl tetrahydromethanopterin (methenyl-H_4MPT, **1**) with H_2 to N^5,N^{10}-methylene tetrahydromethanopterin (methylene-H_4MPT, **2**; $\Delta G^{\circ\prime} = -5.5$ kJ mol^{-1}; Scheme 1).[7] This reaction is involved in the reduction of CO_2 with H_2 to form CH_4 in methanogenic archaea.[8, 9] The enzyme catalyzes a direct hydride transfer from H_2 into the *pro-R* position of **2**.[10] In addition, the enzyme catalyzes an exchange of the hydrogen atom in the *pro-R* position of **2** with the protons of water,[11] a methenyl-H_4MPT-dependent exchange of H_2 with the solvent,[12, 13] and a methenyl-H_4MPT-dependent *ortho/para* H_2 conversion.[14] These properties of metal-free hydrogenase are accounted for in the catalytic mechanism proposed in Scheme 2.[6] The mechanism is based



Scheme 1. The reaction catalyzed by the metal-free hydrogenase. For complete structures of **1** and **2** including sidechains, see references [5, 10].

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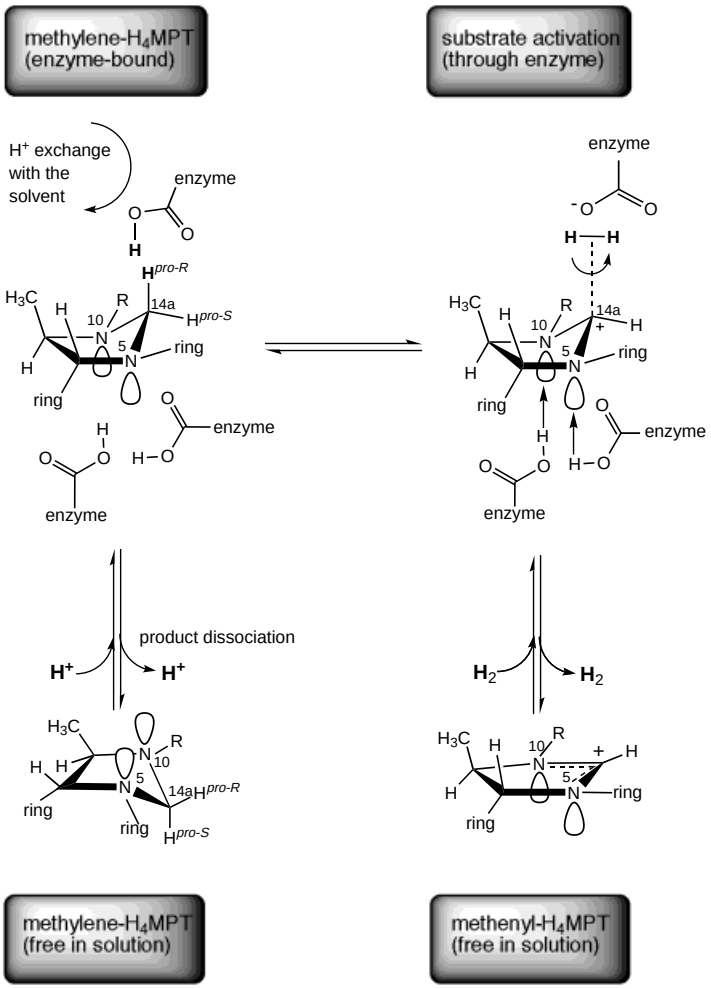


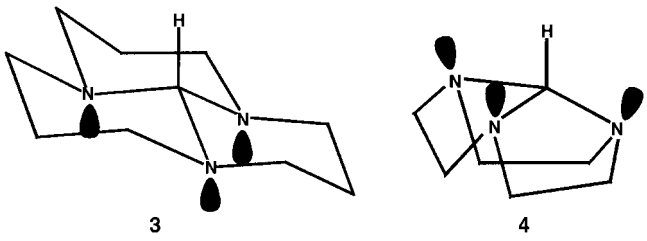
Scheme 2.

on the fact that in superacids carbocations can react directly with H_2 in the absence of other nucleophiles.[15]

In Scheme 2 it is assumed that **1** undergoes a conformational change upon binding to the enzyme. Thus, the conjugational stabilization is eliminated and a cationic center at C14a is created, which in its properties corresponds to a carbocation normally generated only under superacidic conditions. It can be predicted from the stereospecificity of the reaction that in a complex with the enzyme, **2** must be in a conformation in which the *pro-R* C–H bond is antiperiplanar to the free lone electron pairs of the flanking nitrogen atoms. Molecular orbital calculations of the transition state support this mechanism.[16–18]

In Scheme 2 it is further assumed that **2** in aqueous solution, when not bound to the enzyme, adopts a conformation in which the *pro-S* C–H bond is antiperiplanar to the lone electron pairs of the flanking nitrogen atoms. Thus, the conformation must change when the hydrogenation product dissociates from the enzyme. Whereas the conformation of **2** in aqueous solution could not be deduced from analysis of $^1H-{}^1H$ nuclear Overhauser effects (NOEs) and coupling constants,[19] we now provide evidence for this conformation based on the $^1J_{CH}$ coupling constants, the chemical shifts of the *pro-R* and *pro-S* protons, and the stereospecific deuteration of **1**.

Methylene-H_4MPT (**2**) should have similar properties as perhydro-3a,6a,9a-triazaphenalene (**3**),[6, 16] which reacts to the guanidinium salt and H_2 when equivalent amounts of aqueous HBF_4 are added at 110 °C.[20] The C–H bond of the



3 **4**

methine group of **3** is unusually reactive with respect to heterolysis to a carbocation and a hydride.[20–22] The high reactivity is caused by the interaction of the three lone electron pairs of the nitrogen atoms antiperiplanar to the C–H bond. This interaction manifests itself in an unusually low wavenumber for the C–H stretching band in the IR spectrum ($\tilde{\nu} = 2450\ cm^{-1}$;[21, 22] Bohlmann band),[23] a very small $^1J_{CH}$ coupling constant of 141 Hz,[24] and a NMR resonance shifted to high field ($\delta = 2.25 - 2.33$).[21, 22, 24] The latter two can be explained by the conjugation of the nitrogen n orbitals with the σ* orbital of the C–H fragment: The resulting weakening of the C–H bond should lead to a decrease in the coupling constant, and the negative partial charge should cause a high-field shift in the NMR spectrum.[20–22]

In contrast to **3**, the C–H bond of the methine group of perhydro-2a,4a,6a-triazacyclopenta[cd]pentalene (**4**) is much less reactive[20–22] because the methine proton of **4** is in the position *gauche* to the free lone electron pairs of the neighboring nitrogen atoms. A significantly bigger coupling constant $^1J_{CH}$ of 184 Hz,[24] a chemical shift of $\delta = 5.03$,[21, 24] and the absence of a Bohlmann IR band[22, 24] indicate that the C–H bond of the methine group of **4** is stronger. Thus, from these spectroscopic properties the relative orientation of the lone electron pairs to the C–H bond can be deduced.

Table 1 lists coupling constants $^1J_{CH}$ and chemical shifts of the *pro-R* and *pro-S* protons of methylene-H_4MPT (**2**) in aqueous solution. Comparing both parameters with those of **3** and **4** suggests that in aqueous solution **2** is in a conformation in which the *pro-S* proton is more reactive than the *pro-R* proton, and in which the *pro-S* C–H bond is therefore

Table 1. Coupling constants $^1J_{CH}$ and chemical shifts δ of the methylene groups of [^{13}C]methylene-H_4MPT and [^{13}C]methylene-H_4F.[a]

Compound	$^1J_{CH}$ [Hz]	δ
[^{13}C]methylene-H_4MPT		
pro-R	158.0	5.05
pro-S	150.0	4.10
pro-R (M)	157.5	4.98
pro-S (M)	145.5	3.67
[^{13}C]methylene-H_4F		
pro-R	157.0	4.93
pro-S	149.0	3.90

[a] The samples had a concentration of 4 mM and were dissolved in 20 mM formiate buffer (pH 3). (M) indicates samples that were measured in methanol/water (1/1).

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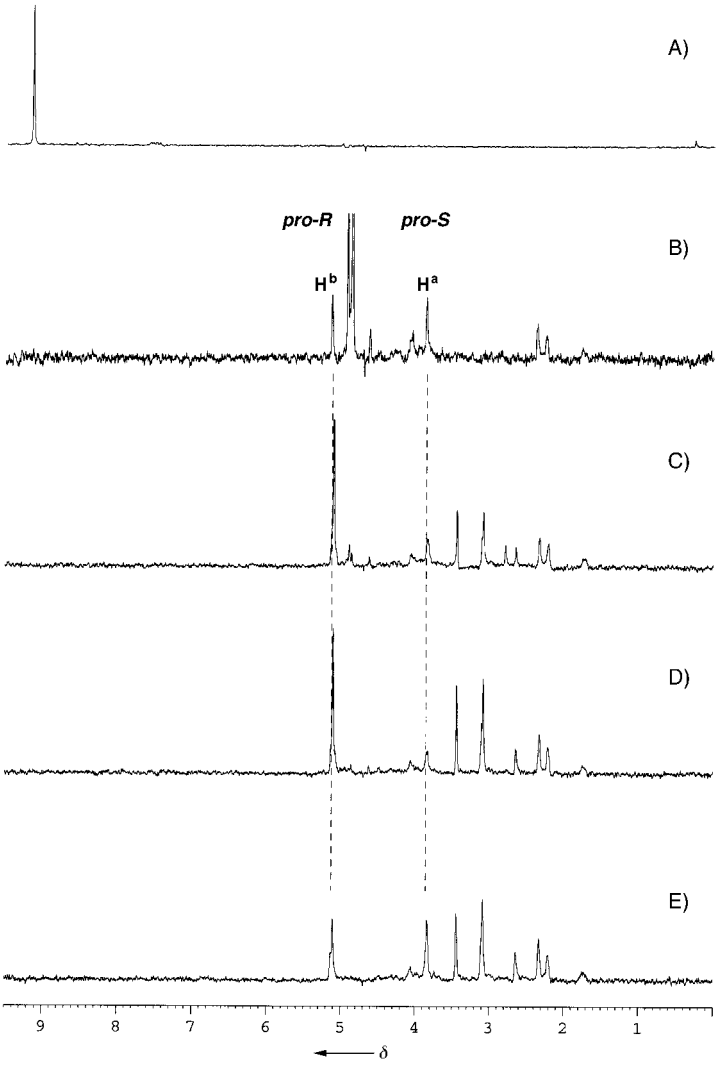

Figure 1. $^1H-^{13}C$ heteronuclear single quantum correlation (HSQC) NMR spectra to monitor the reduction of [14a-^{13}C]methenyl-H_4MPT[10] by $NaBD_4$ to [^{13}C]methylene-H_4MPT. A) Spectrum of a solution of [14a-^{13}C]methenyl-H_4MPT (ca. 2 mM) in 16 mM potassium phosphate (in D_2O, pH 6.0) and 20% acetonitrile. The ^{13}C frequency was set to $\delta = 170$ with $\Delta = 1/(4\,^1J_{CH}) = 1.19$ ms. B) Same sample as in A), but the spectrum was recorded at a ^{13}C frequency of $\delta = 70$. Small amounts of reduced [^{13}C]methylene-H_4MPT are present, as indicated by peaks at $\delta = 5.1$ for the *pro-R* proton H^b and at $\delta = 3.8$ for the *pro-S* proton H^a. C) Same sample as in A), but after addition of $NaBD_4$ (ca. 2 mg). Deuteration occurs selectively at the *pro-S* position, since only the signal intensity of the *pro-R* proton H^b increased. D) Reaction repeated under the same conditions. The acquisition of spectra C) and D) at a ^{13}C frequency of $\delta = 70$ was started approximately 15 min after addition of $NaBD_4$. E) Spectrum of sample from D) approximately 11 h after initiation of the reaction. The intensities of the signals for H^a and H^b have become equal because of epimerization at atom C14a. Experiments in pure water show the same effect. [14a-^{13}C]Methenyl-H_4MPT was produced as described.[19] All 1D $^1H-^{13}C$ HSQC NMR spectra[27–29] were acquired at a ^1H frequency of 600.14 MHz on an AMX600 spectrometer (Bruker) and processed with the program XWINNMR (Bruker) using an exponential window function with 3-Hz line broadening. After 16 dummy scans to allow for preequilibration 256 scans were signal averaged, except for spectrum B (64 scans). The recycle delay was 2 s. A total of 4096 real points were recorded over a spectral width of 6024.1 Hz. The ^{13}C decoupling was achieved by globally optimized alternating phase rectangular pulse (GARP) trains.[30] Spectra were scaled to allow for comparison of absolute peak intensities. All δ values were referenced to the signal for residual HDO in 1D ^1H presat spectra (not shown) at $\delta = 4.70$ and 303 K.

antiperiplanar to the lone electron pairs of the neighboring nitrogen atoms. As a consequence, a significant conformational change must occur when **2** dissociates from the metal-free hydrogenase (Scheme 2), because only the *pro-R* hydrogen atom reacts in the enzyme-catalyzed reaction.[10] In contrast, one would predict that the chemical reduction of methenyl-H_4MPT (**1**) in aqueous solution should lead to the incorporation of a hydrogen atom into the *pro-S* position of **2**, with the methenyl proton going into the *pro-R* position. Indeed, this is what was observed: When **1** was reduced with $NaBD_4$ in D_2O, the deuteride was incorporated into the *pro-S* position of **2** (Figure 1).

Table 1 also shows $^1J_{CH}$ coupling constants and chemical shifts of both methylene protons of N^5,N^{10}-methylene tetrahydrofolate (methylene-H_4F). The similarity between the measured NMR parameters of methylene-H_4F and **2** is evident. A low-field chemical shift correlates with a large $^1J_{CH}$ coupling constant, as does a small $^1J_{CH}$ coupling constant with a high-field chemical shift. It is striking that in this correlation the corresponding methylene protons of **2** and methylene-H_4F occupy almost the same positions (Figure 2). This suggests that the chemical reactivity of the methylene

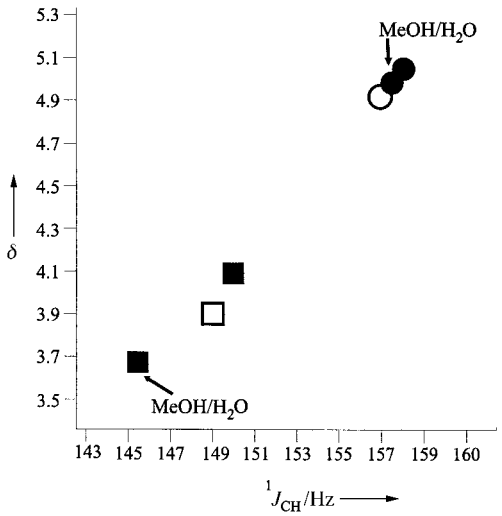

Figure 2. Correlation between chemical shifts δ and $^1J_{CH}$ coupling constants of the methylene protons of methylene-H_4F and methylene-H_4MPT; ■ = *pro-S* proton and ● = *pro-R* proton of methylene-H_4MPT, □ = *pro-S* proton and ○ = *pro-R* proton of methylene-H_4F. Data labeled with MeOH/H_2O were measured in methanol/water (1/1); all other data were recorded in 20 mM formiate buffer (pH 3). The data measured in MeOH/H_2O illustrate that chemical shift and coupling constant correlate regardless of the solvent.

protons is very similar in both compounds. Indeed, in the chemical reduction of N^5,N^{10}-methenyl tetrahydrofolate with $NaBH_4$, the hydride is incorporated into the *pro-S* position as well.[25] Interestingly the enzyme-catalyzed reduction of methenyl tetrahydrofolate with the reduced forms of nicotinamide adenine dinucleotide (NADH) or nicotinamide adenine dinucleotide phosphate (NADPH) to methylene-H_4F also proceeds with reversed stereospecificity.[25, 26]

In summary, the metal-free hydrogenase transfers molecular hydrogen to methenyl-H_4MPT (**1**) with a stereospecificity

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that is reversed from that observed for the reduction of free substrate in solution. The same holds true for the enzyme-catalyzed reduction of methenyl-H_4F with NADH or NADPH as coenzyme. In both cases we can conclude from NMR spectroscopic data that the reactive conformation in the enzyme-catalyzed reaction does not match that in solution. In addition, in the enzyme-catalyzed reaction the product is formed in an energetically unfavorable conformation. This could possibly prevent product inhibition.

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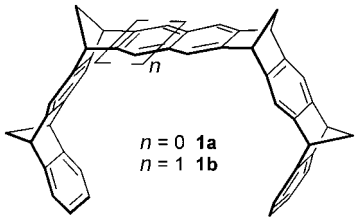
Modeling the Supramolecular Properties of Aliphatic-Aromatic Hydrocarbons with Convex – Concave Topology**

Markus Kamieth, Frank-Gerrit Klärner,* and François Diederich

Dedicated to Professor Emanuel Vogel on the occasion of his 70th birthday

In supramolecular and biological chemistry, for example in the case of molecular recognition, arenes make an important contribution to the formation of superstructures.[1] Their interactions with other arenes ($\pi-\pi$ or arene – arene interactions)[2] and with positively charged ions (cation – π interactions)[3] are particularly common and are used in the design of synthetic receptors. The properties of selective complexation[4] discovered for molecular tweezers of the type **1** and the results known from other receptors with concave – convex topology stimulated the theoretical model studies introduced here.



The molecular tweezers **1a** and **1b** serve as receptors for acceptor-substituted and cationic arenes as well as corresponding aliphatic substrates, whereas electron-rich arenes or anionic compounds are not complexed by them.[4] These experimental results, together with the established solvent dependence, indicate a substantial contribution of electrostatic interaction, as was already described in the electrostatic model for the attractive arene – arene interaction.[2] To find out the extent of electrostatic interaction in the molecular tweezers **1a** and **1b**, we calculated their electrostatic potential surfaces (EPSs) with the semiempirical AM1 method.[5] We thus found a potential which was surprisingly negative for pure hydrocarbons on the concave side of each molecule (most negative potentials: **1a**: -35.48, **1b**: -33.30 kcal mol^{-1}). In contrast, the potentials on the convex sides almost correspond to that of a tetraalkyl benzene such as durene (most negative potential: -25.60 kcal mol^{-1}; Figure 1, Table 1). When analogous calculations are performed for the potential substrates **2** – **6**, the complementary nature of their electrostatic potential surfaces to those of the cavity of the receptors **1a** and **1b** becomes evident.

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