H_2 Activation

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The Crystal Structure of an [Fe]-Hydrogenase–Substrate Complex Reveals the Framework for H_2 Activation**

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Dedicated to Professor Rudolf K. Thauer on the occasion of his 70th birthday

[Fe]-hydrogenase, which is found in hydrogenophilic methanogenic pathway, catalyzes the reversible reduction of methenyltetrahydromethanopterin (methenyl-H_4MPT$^+$) with H_2 to methylene-H_4MPT and a proton by transferring a hydride ion to the *proR* position of the C14a carbon of methylene-H_4MPT (Figure 1 A).[1–3] Crystal-structure and spectroscopic analyses revealed that its iron center is ligated by Cys176-sulfur, two CO, and an sp^2-hybridized nitrogen and an acyl carbon atom of a unique iron-guanylylpyridinol (FeGP)-cofactor (see Figure 1 B).[4–11] The homodimeric protein is built up of two peripheral and one central globular unit, the latter composed of segments of both subunits. It can exist in an open and a closed form (Supporting Information, Figure S1).[10]

[Fe]-hydrogenase catalyzes H^+/H_2-exchange[12–14] and *para*/*ortho*-H_2 conversion[15] but the exchange activities are absolutely dependent on methenyl-H_4MPT$^+$ which indicates that activation of H_2 can only be achieved in the presence of the substrate.[14] Berkessel and Thauer proposed that the cationic methenyl carbon (C14a), generated by binding of the substrate to the enzyme, functions as Lewis acid in the catalysis.[16,17]

A structure-based catalytic mechanism of [Fe]-hydrogenase has to integrate the following conditions, as proposed by Vogt et al.[14] 1) H_2 can interact with the iron center only after the binding of methenyl-H_4MPT$^+$ to the enzyme. 2) In the substrate complex, C14a of methenyl-H_4MPT$^+$ and the iron sit in the vicinity at the active site. 3) The proton derived from the heterolytic cleavage of H_2 is exchanged quickly with protons of bulk solvent.

Herein, we describe for the first time a binary complex crystal structure of [Fe]-hydrogenase with methylene-H_4MPT at 2.15 Å resolution. (The cocrystallized substrate is in its neutral methylene form rather than the cationic methenyl form.) The data meet completely the catalytic mechanism mentioned above[14] and provide new insights into its structural basis. For cocrystallization, we prepared C176A-

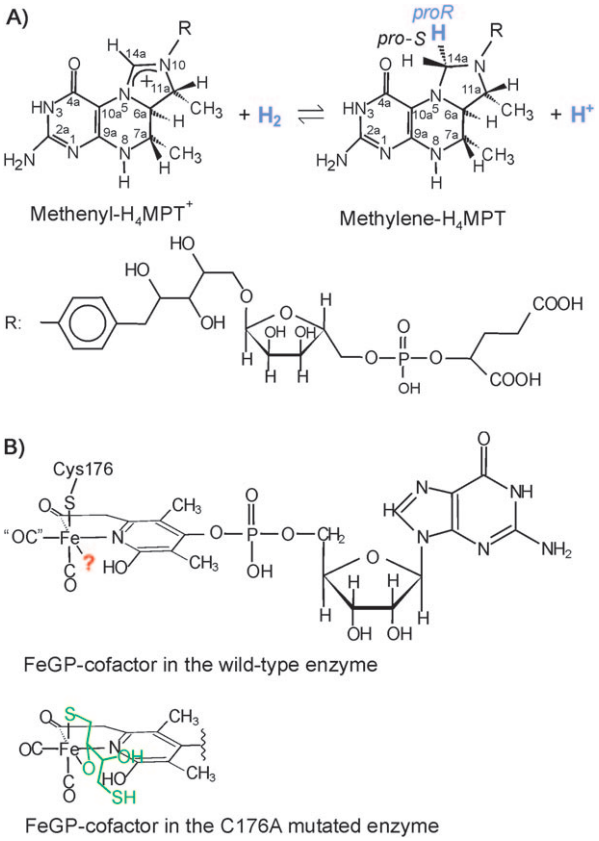

Figure 1. Structures of the substrates and FeGP-cofactor of [Fe]-hydrogenase. A) Reaction catalyzed by [Fe]-hydrogenase. A hydride is reversibly transferred from H_2 into the *proR* position of methenyl-H_4MPT$^+$.[1] B) FeGP-cofactor in the wild-type and C176A-holoenzyme crystals.[10,11] In the C176A holoenzyme, the Cys176-sulfur ligand found in the wild-type is substituted by the 1-thiol group of dithiothreitol (shown in green), which is supplied in the crystallization solution. The 2-hydroxy group of the same dithiothreitol molecule also coordinates to the iron ion. The intrinsic CO-binding site ("CO") *trans* to the pyridinol nitrogen was identified in the crystal structure of the C176A holoenzyme but cannot be unambiguously assigned in the wild-type holoenzyme. The unknown ligand site *trans* to the acyl carbon indicated by "?" could be an alternate CO ligand site.[11]

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mutated [Fe]-hydrogenase from *Methanocaldococcus jannaschii*, reconstituted with FeGP-cofactor. The use of the inactive mutated enzyme could avoid the formation of undesirable substrate/product mixtures, which can hamper the cocrystallization (for the experimental procedure and crystallographic data, see Supporting Information).[8,11] The coordinates and diffraction data have been deposited in the Protein Data Bank with an accession code 3H65 (Supporting Information, Figure S1B).

In the structure (Figure 2) methylene-H_4MPT is clearly visible in the active site cleft as residual electron density. It accurately corresponds to the shape of the pterin, imidazolidine and phenyl rings (head part) but the remaining tail part exposed to bulk solvent is disordered (Figure 2 and Supporting Information S2). The head part is in a relatively "extended" conformation and fit into the active site cleft (Supporting Information, Figure S1B). This conformation is definitely different from the "bent" NMR spectroscopy structure in solution (Supporting Information, Figure S3).[18] In contrast, the overall conformation of the head part resembles that bound to [Fe]-hydrogenase from *Methanothermobacter marburgensis* previously determined by NMR spectroscopy[18,19] although a closer look reveals functionally relevant differences, in particular, at N5 and N10 of the imidazolidine ring. In the crystal structure both atoms are virtually in planar conformations (Supporting Information, Figure S3) whereas in the NMR structure the conformations of N5 and N10 are in a tetrahedral sp^3-hybridized form, in which lone-pair electrons of N5 and N10 lie at the *proR* and *proS* side, respectively. The reason for this difference might be that methylene-H_4MPT in the crystal structure is not present in an activated conformation, perhaps caused by the missing interactions to its *Re*-side in the open enzyme form. In contrast, the NMR structure of methylene-H_4MPT was presumably derived from a closed and active holoenzyme-substrate conformation, and continuous turnover could be detected by following methylene-H_4MPT formation after dissociation (see below).

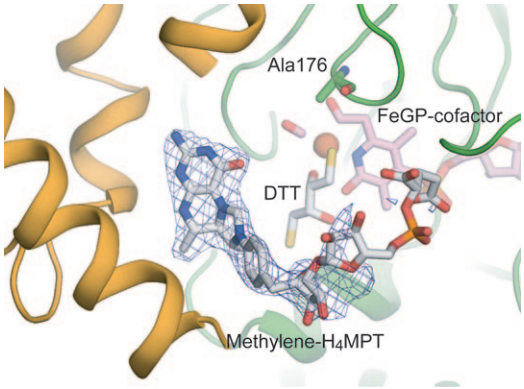

Figure 2. Methylene-H_4MPT in C176A-mutated [Fe]-hydrogenase. The F_o-F_c omit electron-density map (2.8 σ level in blue) around methylene-H_4MPT. The tail part of methylene-H_4MPT is disordered. FeGP-cofactor and methylene-H_4MPT are depicted as color-coded stick-models with the carbon atoms in pink and in gray, respectively, N blue, O red, P orange. Dithiothreitol (DTT) carbon atoms are shown in gray. The peripheral unit and the central unit are shown in green and orange, respectively. For clarity the helix region (251–271) was omitted. Fe red sphere.

The transition from a planar conformation at N5 and N10 of methylene-H_4MPT observed in the crystal structure to the active non-planar conformations in the NMR structure might be induced by a rotation of the phenyl ring of methylene-H_4MPT upon cleft closure (see below). This hypothesis was inspired by Bartoschek et al. who, from NMR spectroscopic measurements, derived that the rotation angle of the phenyl ring could affect the conformations of N10.[19] Previously, it was proposed that inversion of the lone electron pair on N10 and thereby activation of the *proR* hydrogen atom might be facilitated by protonation of the lone electron pair on N5 and on N10 by carboxy groups.[16] However, no carboxy group is present in the vicinity of the imidazolidine ring both in the open and closed forms.

The overall structure of the binary complex is in the open conformation (Figure 3 A and Supporting Information S1). The open cleft between the central and peripheral units

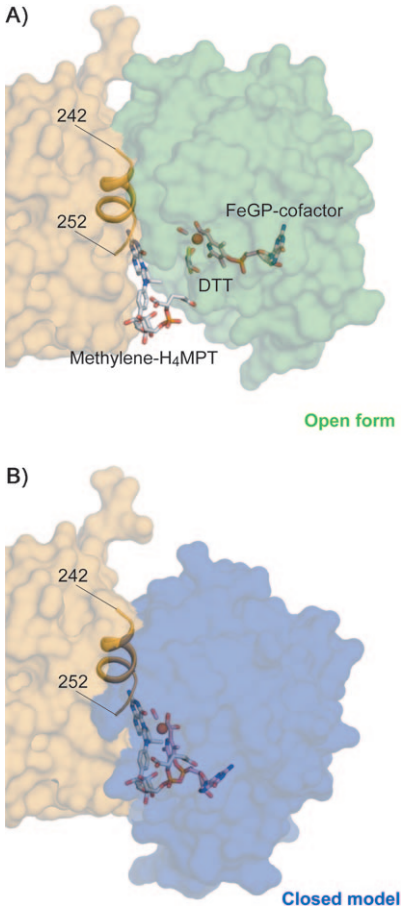

Figure 3. The conformational forms of [Fe]-hydrogenase. A) Molecular surface representation of the binary complex in the open form as experimentally determined and B) of the modeled structure containing a closed active-site cleft. Colors as for Figure 2. In the open form the *Si*-face and the front side of the head part of methylene-H_4MPT is attached to the central unit and a few residues of the hinge region (242–252) whereas its *Re*-face and the tail part are solvent exposed and, surprisingly, not in contact to any polypeptide residue. In (B), DTT is omitted for clarity.

implies a distance of 9.3 Å between the iron and the C14a of the substrate which is too long for transferring a hydride ion even when H_2 binds in an end-on conformation. For modeling of a catalytically productive conformation we used the apoenzyme of [Fe]-hydrogenase from *M. jannaschii* which was crystallized in a closed form.[9] The structures of the peripheral and central units of the "closed" apoenzyme and of the "open" binary complex are almost identical as indicated by root mean square (r.m.s.) deviations of 0.58 Å and 0.42 Å, respectively, implicating a large-scale rigid-body movement between both globular units. Therefore, the closed form was modeled by superimposing the peripheral unit (1–241) and the central unit (253–345) of the binary complex onto those of the apoenzyme (Figure 3).

In the modeled closed form of the binary complex the active site is located in a cavity which implicates the formation of new interfaces between the peripheral and central units. The cavity is accessible to bulk solvent through a narrow hydrophobic channel with a length of approximately 10 Å and a diameter of approximately 4 Å (Supporting Information, Figure S4) and might be used by H_2.

In the closed form, besides dithiothreitol (DTT) bound to the iron, only a few atoms of the FeGP cofactor interfere with the atoms of methylene-H_4MPT. We postulate that the possible orbital overlap would disappear upon the conformational change of methenyl-H_4MPT$^+$ in the activated form. The iron center of the FeGP-cofactor lies in the closed form in front of the *Re*-face of methylene-H_4MPT thereby contacting C14a in 3 Å. The hydride-accepting C14a is next to the iron coordination site *trans* to the acyl carbon of FeGP-cofactor, which strongly suggests this site as the binding position for H_2. Consequently, the previous solvent binding site in the wild-type holoenzyme positioned *trans* to the pyridinol nitrogen atom[10] has to be the iron coordination site of the second intrinsic CO ligand as already found in the C176A-holoenzyme structure (Figure 1B and Supporting Information S5).[11]

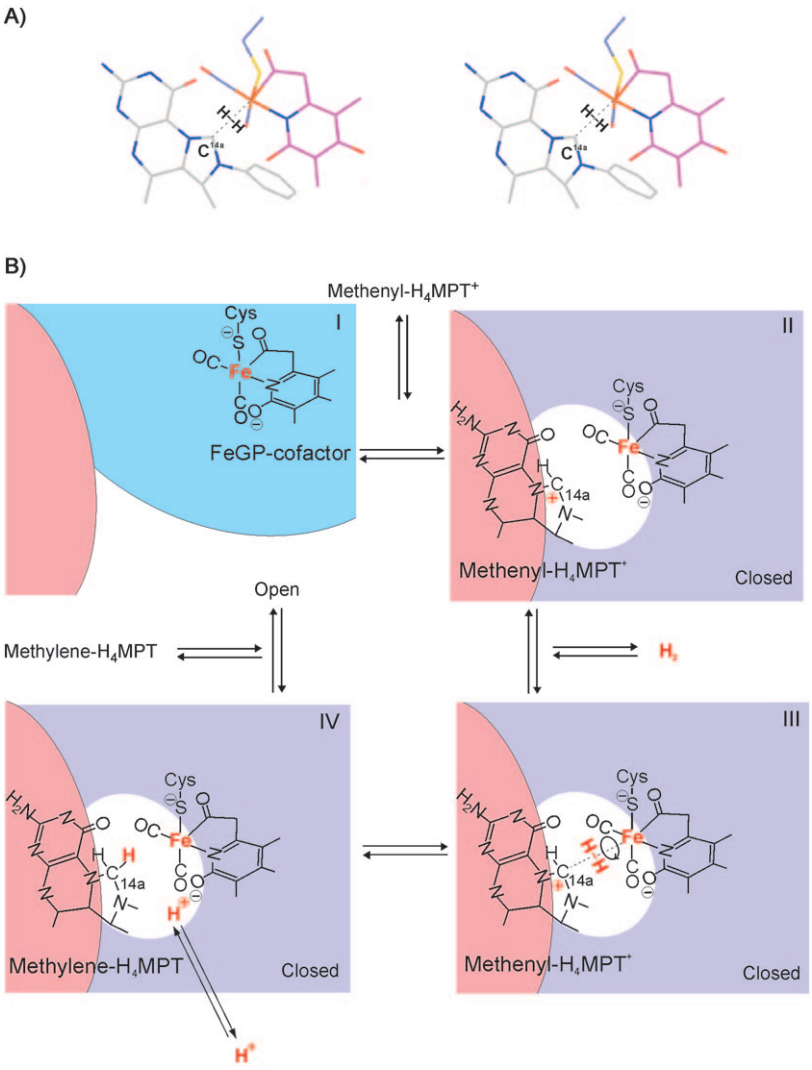

Figure 4. Proposed catalytic mechanism of [Fe]-hydrogenase involving the open/closed conformational transition. A) Stereoview of the predicted framework of the H_2-activating site. For the color codes, see Figure 2. The conformation of methenyl-H_4MPT$^+$ was derived from the NMR spectroscopic data of methylene-H_4MPT.[19] B-I) Open form. B-II) Upon binding of methenyl-H_4MPT$^+$, the active-site cleft is closed. The hydroxy group of the pyridinol ring could interact with the N10 of methylene-H_4MPT, which may modify the properties of the pyridinol.[20] B-III) H_2 reaches the active site through the hydrophobic channel and binds side-on to the iron. B-IV) The carbocationic C14a of methenyl-H_4MPT$^+$ accepts the hydride on its *Re*-face and thereby generates methylene-H_4MPT. The proton quickly exchanges with protons in bulk solvent via a proton relay pathway. The peripheral and central units are shown in blue/purple and pink, respectively. The white oval in II–IV indicates the active site cavity.

The proposed catalytic mechanism of [Fe]-hydrogenase is shown in Figure 4. The catalytic cycle is initiated by binding of methenyl-H_4MPT$^+$ to the open form which would trigger the closure of the cleft (Supporting Information, Figure S6) and might change thereby its conformation resulting in an increased carbocationic character at C14a (Figure 4A and B-II).[16,17,21] Concomitantly, the FeGP-cofactor might also be activated[20] and H_2, supplied through the hydrophobic channel to the active site of the closed form, is captured at the "open" coordination site *trans* to the acyl carbon of the iron center (Figure 4B-III). The oxidation state of the iron is proposed to be low-spin Fe^{II}.[22,23] The H_2 molecule probably binds side-on to the iron, becomes somewhat polarized and heterolytically cleaved by the adjacent C14a carbocation acting as a Lewis acid as proposed previously.[14,16–18] The hydride is accepted by methenyl-H_4MPT$^+$ and the proton by a base which might be the deprotonated form of the Cys176 thiol or pyridinol hydroxy group. The use of Cys176 thiolate as a proton acceptor would be reminiscent of the situation in [NiFe]-hydrogenase in which the terminal cysteine-thiolate ligand on the nickel is discussed as a base.[24,25] Alternatively,

the pyridinol hydroxylate group is an attractive candidate because of its direct contact to the potential H_2-binding site and its possible interaction with the side chain of His14 (Supporting Information, Figure S5). A H14A exchange reduces the activity of the wild-type enzyme to less than 1%.[10]

In the modeled closed form of the C176A-holoenzyme-substrate complex, the *Re*-face of methenyl-H_4MPT^+ contacts the iron center of the FeGP-cofactor, thus creating a dinuclear catalytic center composed of a carbocation and low-spin iron(II) (Figure 4A). The dinuclear C-Fe arrangement of [Fe]-hydrogenase resembles to that of the dinuclear metal center of [NiFe]- and [FeFe]-hydrogenases. The applications of the dinuclear center similarly arranged as well as the related low-spin iron complex structures in all three types of hydrogenases suggest a convergent evolution of the H_2-activation machinery. The only variable is the first cation which can be a nickel, an iron, or a carbon. The coordination geometry of the closed binary complex can be used as starting model for calculations and for designing model compounds mimicking the dinuclear center.

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