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Crystal Structures of [Fe]-Hydrogenase in Complex with Inhibitory Isocyanides: Implications for the H_2-Activation Site**

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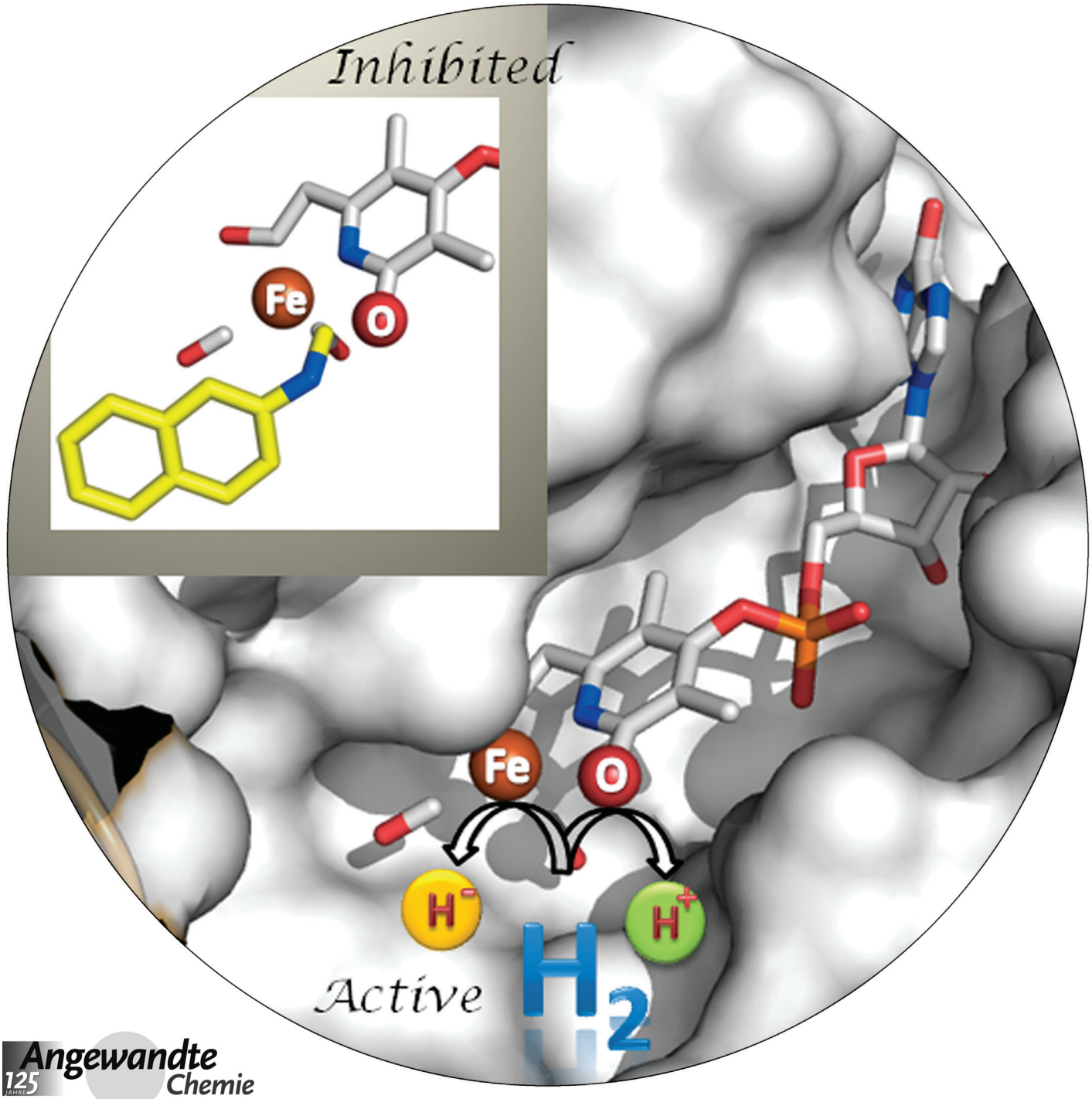

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[Fe]-hydrogenase (Hmd), an enzyme involved in the archaeal methane forming pathway, catalyzes the reversible hydride transfer from H_2 to methenyltetrahydromethanopterin using a unique iron guanylylpyridinol (FeGP) as a cofactor (Supporting Information, Figure S1).[1] The FeGP cofactor contains a single iron atom in the low-spin Fe^{II} oxidation state.[2] This iron atom is coordinated by a cysteine sulfur, two *cis*-CO ligands, and the pyridinol nitrogen and acyl carbon of the FeGP cofactor (Figure 1).[3] H_2 was proposed to bind the

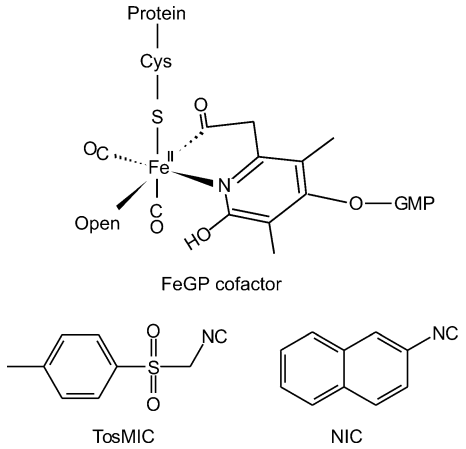

Figure 1. Formulas of the FeGP cofactor (enzyme-bound form) and the isocyanides *p*-toluenesulfonylmethylisocyanide (TosMIC, $K_i = 2$ nM) and 2-naphthylisocyanide (NIC, $K_i = 10$ nM). In the native enzyme crystal structure, a solvent molecule is bound at the "Open" site.[3a] The crystal structure of the enzyme suggested a pyridinol rather than a pyridone tautomeric form for the FeGP cofactor.[3c,e]

empty sixth coordination site of the iron, positioned *trans* to the acyl group, based on indirect evidence from the crystal structures.[3b] Density functional theory (DFT) calculations also suggest that H_2 binds to the proposed iron site and that the pyridinol oxygen stabilizes a catalytic step, in which H_2 is captured between the iron and the pyridinol oxygen.[4] In contrast to [NiFe]- and [FeFe]-hydrogenases, [Fe]-hydrogenase is effectively inhibited by isocyanides such as *p*-toluenesulfonylmethylisocyanide (TosMIC; $K_i = 2$ nM) and 2-naphthylisocyanide (NIC; $K_i = 10$ nM; chemical formulas Figure 1; kinetics data Figure S2). Previous kinetics studies have suggested that H_2 and isocyanides bind to the same ligation site.[5] Herein, we report the iron-ligation structure of the enzyme inhibited with TosMIC or NIC analyzed by X-ray crystallography and X-ray absorption spectroscopy (XAS). The structures of the [Fe]-hydrogenase complex with TosMIC and NIC (at 2.5 Å and 2.2 Å resolution, respectively) revealed that the isocyanides were bound to the proposed H_2-binding iron site *trans* to the acyl-carbon ligand. Unexpectedly, the iron-ligating isocyanide carbon was also covalently bound to the pyridinol hydroxy oxygen of the FeGP cofactor. This finding suggested a greater importance of this group in molecular hydrogen activation than previously thought.

The structures of the isocyanide-bound [Fe]-hydrogenase from *Methanothermobacter marburgensis* were very similar to that from *Methanocaldococcus jannaschii* (Supporting Information, Figures S3–S5).[3b,c,6] In both enzyme/TosMIC (PDB code: 4JJG) and enzyme/NIC (PDB code: 4JJF) structures of *M. marburgensis*, the FeGP cofactor was unambiguously identified in the electron density at its known binding site in the N-terminal domain (Figure 2; Figure S4). As predicted, the isocyanides were bound to the coordination site *trans* to the acyl carbon. However, coordination between iron and the isocyano carbon was not possible without a severe collision with the pyridinol hydroxy oxygen suggesting a covalent bond between the isocyano carbon and the hydroxy oxygen. Subsequent refinement taking into account a covalent bond produced a model without significant residual electron density. For both TosMIC and NIC, the obtained distance between the isocyano carbon and the hydroxy oxygen of the pyridinol ring is approximately 1.5 Å (Figure 2; Figure S8). The distance between the iron atom and the isocyano carbon atom is approximately 1.9 Å for both TosMIC and NIC, which is in agreement with the value calculated from XAS measurements (Table 1; Figures S6,S7). The binding mode of TosMIC and NIC is identical for the isocyanide group, however, the conformations of their substituents were clearly distinguished. Only TosMIC can form a hydrogen bond to the

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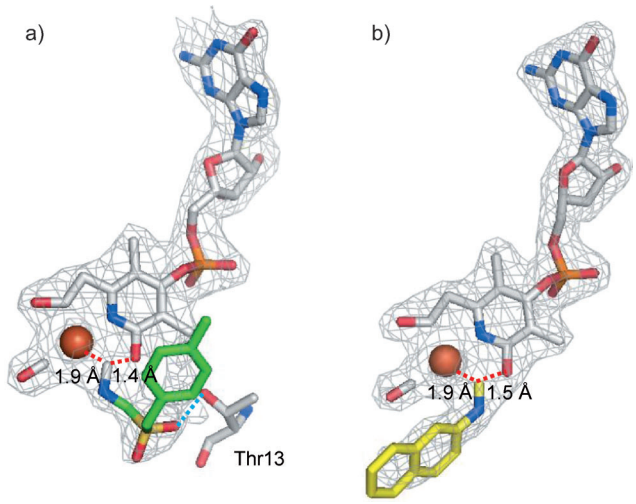

Figure 2. Binding of isocyanide inhibitors to the FeGP cofactor of [Fe]-hydrogenase. a) TosMIC and b) NIC with FeGP cofactors as stick models. The C atoms of the FeGP cofactor, TosMIC, and NIC are drawn in light gray, green, and yellow; O red, N blue, S yellow, Fe brown sphere. Covalent bonds between FeGP and the inhibitors are red dashed lines. Potential hydrogen bonds between the sulfonyl O atom of TosMIC and the hydroxy group of Thr13 is a blue dashed line. The $2F_o-F_c$ electron density maps (in gray mesh) are contoured at 1.0 σ. Maps of the NIC complex at higher σ values are shown in Figure S8.

Table 1: Structural parameters extracted from the EXAFS refinement for *M. marburgensis* [Fe]-hydrogenase.[a]

EXAFS refinement parameters[b]							
n	Fe	L	*R* [Å]	$2\sigma^2$ [Å^2]	R_{C-X}(Å) [Å]	E_F [eV]	Φ (×10^3)
TosMIC-inhibited							
2	Fe	C[c]	1.77(1)	0.005(3)	1.18(2)	−18(1)	3.123
1	Fe	C[d]	1.96(1)	0.007(2)	1.31(5)		
1	Fe	C[e]	1.96(1)	0.007(2)			
1	Fe	N	1.96(1)	0.007(2)			
1	Fe	S	2.36(1)	0.008(2)			
2	Fe	O[c]	2.95(1)	0.010(2)			
1	Fe	N[d]	3.27(4)	0.010(2)			
Cyanide-inhibited							
2	Fe	C[c]	1.775(4)	0.0068(7)	1.146(7)	−18.1(3)	0.158
1	Fe	C[d]	1.993(5)	0.0208(5)	1.149(9)		
1	Fe	C[e]	1.993(5)	0.0208(5)			
1	Fe	N	1.993(5)	0.0208(5)			
1	Fe	S	2.353(4)	0.0130(7)			
2	Fe	O[c]	2.921(3)	0.0036(4)			
1	Fe	N[d]	3.142(4)	0.0036(4)			
Active state							
2	Fe	C[c]	1.77(1)	0.009(3)	1.15(2)	−9.6(4)	0.117
1	Fe	C[e]	1.85(2)	0.004(4)			
1	Fe	N	2.043(8)	0.012(1)			
1	Fe	O	2.043(8)	0.012(1)			
1	Fe	S	2.314(3)	0.0047(6)			
2	Fe	O[c]	2.925(3)	0.0075(4)			

[a] The data of the last two samples were obtained from Korbas et al.[16] [b] The numbers (*n*) of ligand atoms (L) to the iron, their distance to the iron (*R*), the respective Debye-Waller factor ($2\sigma^2$), the C–O or C–N bond length (R_{C-X}), the Fermi energy for all shells (E_F), and the fit index (Φ) indicating the quality of the fit are shown. [c] Carbon or oxygen atoms of CO, [d] carbon or nitrogen atoms of the isocyanide group, [e] carbon atom of the acyl group.

protein by its sulfonyl oxygen to Thr13 (Figure 2), which explains its lower K_i value compared to other isocyanides.[5] The B-factor of the bound isocyanide increases from the iron-ligating carbon towards the toluene and naphthalene rings of TosMIC and NIC from 33 Å^2 to 91 Å^2 and from 39 Å^2 to 79 Å^2, respectively, indicating an increasing flexibility with the distance to the iron. The positions (but not an accurate shape) of the peripheral *p*-tolyl and naphthyl groups were visible in the $2F_o-F_c$ maps of the [Fe]-hydrogenase/TosMIC and [Fe]-hydrogenase/NIC structures (Figure 2). The B-factors of the FeGP cofactor are 35 Å^2 and 37 Å^2 (Table S1), respectively, such that the occupancies of the inhibitors are approximately 80–100%.

The high affinity (K_i in the nanomolar range) of isocyanides to [Fe]-hydrogenase could be due to the bridging bond between the isocyano carbon and both the iron and the pyridinol hydroxy oxygen. The bridging bond might be mechanistically accomplished, by forming initially a coordination bond between the negatively charged isocyano carbon and the Fe^{II} atom at its vacant coordination site *trans* to the acyl group. Subsequently, the electrophilic isocyanide carbon is attacked by the pyridinol hydroxy oxygen to form an imine (Figure 3). Owing to the conjugated pyridinol system and the

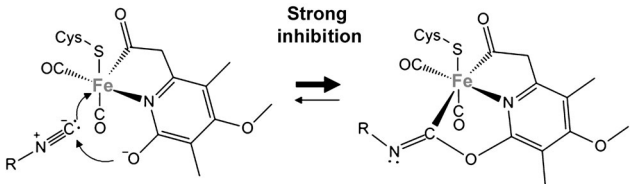

Figure 3. Mechanism of covalent bond formation between the isocyano carbon and both the iron and the pyridinol hydroxy oxygen of the FeGP cofactor.

adjacent His14 and Thr13 side chains the hydroxy group is presumably deprotonated which increases its nucleophilicity. The nucleophilic addition reaction further implicates a conversion of a C–N triple bond to a C–N double bond and subsequent acceptance of the electron pair by the nitrogen (Figure 3). A related nucleophilic addition reaction to an imine is not possible for cyanide because its nitrogen is too weak of an electron acceptor. Therefore, cyanide is only ligated to the iron, which is reflected in a high K_i value (200 μM).[7] The reversibility of the inhibition despite the enzyme/isocyanide adduct might be correlated to the severe distortion of the five-membered ring (Figure 3), which results in destabilization of the FeGP-cofactor/inhibitor complex.

A covalent bond between a metal-coordinated isocyano carbon and a hydroxy oxygen is novel in proteins but precedents exist in chemistry. For example, in the Cu_2O catalyzed cyclization of isocyanobenzyl alcohols the isocyano group is coordinated to the Cu^I species and a subsequent nucleophilic addition of the hydroxy group to the isocyano carbon yields a metalated oxazoline after deprotonation.[8] Nucleophilic attack at the isocyano carbon of Fe^{II} complexes is also known.[9] Isocyanide binding to metals in metalloproteins (without forming an additional bond between the isocyanide carbon and a hydroxy oxygen) is well established.[10] In the crystal structures of *n*-butyl isocyanide-bound

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hemoproteins, P450cam[10b] and myoglobin,[10a] the isocyanide carbon is ligated to the heme iron with a distance of 1.9 Å as found in the [Fe]-hydrogenase/isocyanide complexes. The Fe–C–N angle for P450cam was 159° and for myoglobin was 143°, this deviates from the expected linearity (180°)[11] because of a steric effect in their distal heme pockets.[10b] In contrast to their *sp* hybridization, the Fe–C–N angles of the isocyanide-inhibited [Fe]-hydrogenase are 119° (TosMIC) and 125° (NIC), which confirmed that the isocyanide carbon bound to [Fe]-hydrogenase is sp^2 hybridized.

The mode of isocyanide-binding to [Fe]-hydrogenase clearly demonstrated that H_2 is bound to the iron site *trans* to the acyl ligand and that the pyridinol hydroxylate group affects the chemistry at the H_2 binding site. Thus, most likely, H_2 is not only activated by a specifically ligated iron but also by the pyridinol hydroxy group. The optimally positioned hydroxylate group serves in the reaction with the isocyano carbon as a nucleophile and in H_2-activation as a "pendant" base. In [FeFe]-hydrogenases, H_2 is proposed to be activated by binding between an iron site and the N atom of the dithiol bridge ligand.[12] Recently, Pickett and collaborators have proposed an Fe-H transition state stabilized by an intramolecular Fe–H···O (pyridinol) hydrogen bond analogous to a tungsten-hydride compound, which is hydrogen-bonded to a carboxylate O atom of an intrinsic ligand. The formed five-membered cyclic structure is composed of W, carboxylate O, carboxylate C, carboxylate O, and H atoms.[13] Berkessel and von der Höe synthesized cyclopentadienone iron complexes that cleave H_2 and use a cyclopentadienone O atom for catalysis.[14] Our finding is of general interest for the biomimetic H_2-activation field as model compounds mimicking the FeGP cofactor and for designing novel base-metal catalysts containing pyridinol as pendant bases. To date, the C2-position of the ring of the FeGP cofactor model compounds has always been substituted with a methyl/methoxy/hydroxymethyl group or a hydrogen rather than with a hydroxy group.[15] We, therefore, strongly recommend the introduction of a C2 pyridinol-hydroxy group for improving model compounds functionally mimicking [Fe]-hydrogenase.

Experimental Section

[Fe]-hydrogenase was purified from *Methanothermobacter marburgensis*. The protein-isocyanide complexes were crystallized at 8 °C using the sitting drop vapor diffusion method. X-ray diffraction data were collected at 100 K at the Swiss-Light Source (Villigen, Switzerland). The structures were determined by molecular replacement. X-ray absorption spectra at the Fe K-edge were recorded in transmission mode at Wiggler station 7–3 (SSRL, Menlo Park, CA, USA) equipped with a Si(220) double crystal monochromator, a focusing mirror, and a 30-element Ge solid-state fluorescence detector (Canberra). For experimental details, see the Supporting Information.

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