The [Fe]-hydrogenase (Hmd) is a metalloenzyme with a novel iron binding motif

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Hydrogenases are enzymes that catalyze the reversible oxidation of molecular hydrogen [1]. Their structure and catalytic mechanism are of considerable applied interest as models for the development of efficient catalysts for hydrogen fueled processes. Despite intensive efforts, however, the understanding of how hydrogenases react with H₂ is only in its infancy. Two of the three known types of hydrogenases are iron-sulfur proteins that contain - besides one or several iron-sulfur clusters - a dinuclear metal center, either [NiFe] or [FeFe], which was shown to be the site of H₂ reaction. Both dinuclear hydrogenases catalyze the reversible formation of 2e⁻ and 2H⁺ from H₂. The third type of hydrogenase, [Fe]-hydrogenase does not contain any iron-sulfur cluster and has been found only in some methanogenic archaea. It shows no sequence similarity to [NiFe]- and [FeFe]-hydrogenases [2]. Its systematic name, H₂-forming methylene-H₄MPT dehydrogenase (Hmd), indicates that the enzyme catalyzes the dehydrogenation of methylene-H₄MPT to methenyl-H₄MPT⁺. Here H₄MPT is the abbreviation for tetrahydromethanopterin, which is a structural and functional analog of tetrahydrofolate.

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\text{H}_2 + \text{Methenyl-H}_4\text{MPT}^+ \rightleftharpoons \text{Methylene-H}_4\text{MPT} + \text{H}^+ \quad \Delta G^{\circ'} = -5.5 \text{ kJ/mol}
\]

The Hmd holoenzyme is composed of two identical subunits of molecular mass 38 kDa and harbors 2 mol of iron per homodimer [3]. Each iron ion in Hmd is tightly bound to guanlyl pyridone [4, 5]. X-ray absorption spectroscopy (XAS) on the active, as isolated enzyme from *Methanothermobacter marburgensis* (mHmd) and from active, reconstituted enzyme from *Methanocaldococcus jannaschii* (jHmd) revealed the presence of mononuclear iron with two CO, one sulfur and one or two N/O in coordination distance. In jHmd the single sulfur ligand is most probably provided by Cys176 as deduced from a comparison of the activity and of the X-ray absorption- and Mössbauer spectra of the enzyme mutated in any of the three conserved cysteines. In the isolated Hmd cofactor two CO, one S and two N/O atoms coordinated the iron, the sulfur ligand being most probably provided by mercaptoethanol which is absolutely required for the extraction of the iron-containing cofactor from the holoenzyme and for the stabilization of the extracted cofactor. The iron ion in Hmd can bind one inhibitor molecule, either extrinsic CO or cyanide [6], as indicated by IR spectroscopy [7]. In active mHmd holoenzyme the number of iron ligands increased by one when one of the Hmd inhibitors (CO or KCN) were present, indicating that in active Hmd the iron contains an open coordination site, which is proposed to be the site of H₂ interaction [8].

The finding that the mononuclear iron active site of Hmd contains - besides 2 CO - also a sulfur ligand is remarkable. The low spin iron in the dinuclear centers of [NiFe]-hydrogenases and [FeFe]-hydrogenases is also coordinated by CO and sulfur ligands. The presence of only one cysteine residue harboring the metal-containing cofactor in Hmd resembles especially the structure of the catalytic site of [FeFe]-hydrogenases, where the dinuclear Fe-Fe center is as well bound to the protein through only one cysteic sulfur [9]. Considering that the three types of hydrogenases are
phylogenetically not related the finding that they have a low spin, low oxidation state iron ion complexed by CO and S in common must be mechanistically meaningful.

Figure 3: Structural models of the iron site in Hmd holoenzyme and iron-containing Hmd cofactor derived by XAS. (A) active Hmd from *M. marburgensis* (mHmd); (B) CO-inhibited mHmd; (C) KCN-inhibited mHmd; (D) reconstituted active Hmd from *M. jannaschii* (jHmd-wildtype); (E) reconstituted active jHmd C10A or C250A mutants; and (F) iron-containing Hmd cofactor. For structure of the organic part of the iron-containing Hmd cofactor see [5].

References