The Mononuclear Iron Active Site of the Iron-Sulfur-Cluster-Free Hydrogenase (Hmd)

Rudolf K. Thauer

Department of Biochemistry, Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

Abstract*:* The iron-sulfur-cluster free-hydrogenase (Hmd) from methanogenic archaea catalyzes the reversible reduction of methenyltetrahydromethanopterin (H_4MPT^+) with H_2 to methylenetetrahydromethanopterin (methylene-H_4MPT) in a stereospecific hydride transfer reaction. The homodimeric enzyme is reversibly inhibited by CO and by cyanide. Hmd contains per 38 kDa subunit one mol of iron, which is associated with a light sensitive cofactor that can be extracted. Active enzyme can be reconstituted from apoprotein and the iron-containing cofactor. We report here on IR-, Mössbauer- and XA spectra of the holoenzyme and of the isolated cofactor. The results indicate that the active site of Hmd harbors a mononuclear Fe center in a low spin and low oxidation state. The iron is complexed by 2 CO, 1 S and 1 N/O and has an open coordination site for the binding of extrinsic CO or of cyanide. Interaction of H_2 with the iron center is dependent on the presence of methenyl-H_4MPT^+ as revealed by IR spectroscopy , which is consistent with the finding that Hmd catalyzes an H_2/H^+ exchange or the conversion of *para* H_2 into *ortho* H_2 only in the presence of the carbocation.

Lyon, E.J., Shima, S., Buurman, G., Chowdhuri, S., Batschauer. A., Steinbach, K. & Thauer R. K.: UV-A/blue-light inactivation of the 'metal-free' hydrogenase (Hmd) from methanogenic archaea. The enzyme contains functional iron after all. *Eur. J. Biochem.* **271**, 195-204 (2004).

Shima, S., Lyon, E.J., Sordel-Klippert, M., Kauss, M., Kahnt, J., Thauer, R. K., Steinbach, K., Xie, X., Verdier, L. & Griesinger, C.: The cofactor of the iron-sulfur cluster free hydrogenase Hmd: structure of the light-inactivation product. *Angew. Chem. Int. Ed.* **43**, 2547-2551 (2004).

Lyon, E.J., Shima, S., Boecher, R., Thauer, R. K., Grevels, F. W., Bill, E., Woseboom, W. & Albracht, S. P. J.: Carbon monoxide as an intrinsic ligand to iron in the active site of the iron-sulfur-cluster-free hydrogenase H_2-forming methylenetetrahydromethanopterin dehydrogenase as revealed by infrared spectroscopy. *J. Am. Chem. Soc.* **126**, 14239-14248 (2004).