DOI: 10.1002/ejic.201001255

Hydrogenases and the Global H_2 Cycle

Rudolf Kurt Thauer*[a]

Keywords: Hydrogen / Nickel / Iron / Sulfur / Carbonyl ligands / Cyanide ligands

This essay for EurJIC's cluster issue on hydrogenases introduces the reader into the global H_2 cycle and the structure and function of the three different types of hydrogenase involved in proton reduction and H_2 oxidation. The mechanism, biosynthesis, evolution, and application of hydrogenases are shortly addressed, which are topics dealt with in more depth in the individual contributions.

Estimates are that approximately 10% of the annual global net primary production of biomass from CO_2 via oxygenic photosynthesis is re-oxidized to CO_2 with sulfate or converted to CO_2 and methane in anoxic environments such as marine and freshwater sediments (Figure 1). H_2 is an important intermediate in this process. It is formed from biomass (mainly cellulose) by anaerobic bacteria, protozoa, fungi, and algae and consumed mainly by sulfate-reducing bacteria and by methanogenic archaea. The H_2 steady state concentration is generally very low, near 1 Pa when sulfate is available as electron acceptor and approximately 10 Pa when CO_2 reduction to methane predominates. Despite these low concentrations, the amounts of H_2 formed and consumed add up globally to 0.3 Gt H_2 annually, approximately half of it being used for methanogenesis from CO_2.[1–4]

There are thermodynamic and mechanistic reasons for the low H_2 concentrations in the anoxic environments. The oxidation of glucose to 6 CO_2 and 12 H_2 is associated with a free energy change $\Delta G^{\circ\prime}$ = +3 kJ/mol under standard conditions [$p(H_2)$ = 10^5 Pa], and, for mechanistic reasons, with the phosphorylation of 4 ADP, for which at least 4 × –60 kJ/mol are required under the physiological conditions of the cell.[5] Glucose conversion to 6 CO_2 and 12 H_2 is exergonic by –240 kJ/mol only if the $p(H_2)$ is lower than 10 Pa. At 10 Pa, the free energy change associated with CH_4 formation from 4 H_2 and CO_2 ($\Delta G^{\circ\prime}$ = –131 kJ/mol) is –40 kJ/mol and still sufficient to support the growth of methanogens that synthesize 0.5 mol ATP per mol CH_4. Because H_2S formation from 4 H_2 and sulfate ($\Delta G^{\circ\prime}$ = –152 kJ/mol) is by –21 kJ/mol more exergonic than CH_4 formation from 4 H_2 and CO_2, the free energy change associated with this reaction still allows the synthesis of ATP at H_2 partial pressures as low as 1 Pa, which is why sulfate reducers generally outcompete methanogens in H_2 consumption.

The standard redox potential E_o' of the $2H^+/H_2$ couple is –414 mV. At $p(H_2)$ = 10 Pa the redox potential is approximately –300 mV and at $p(H_2)$ = 1 Pa approximately –270 mV. Thus, this is the

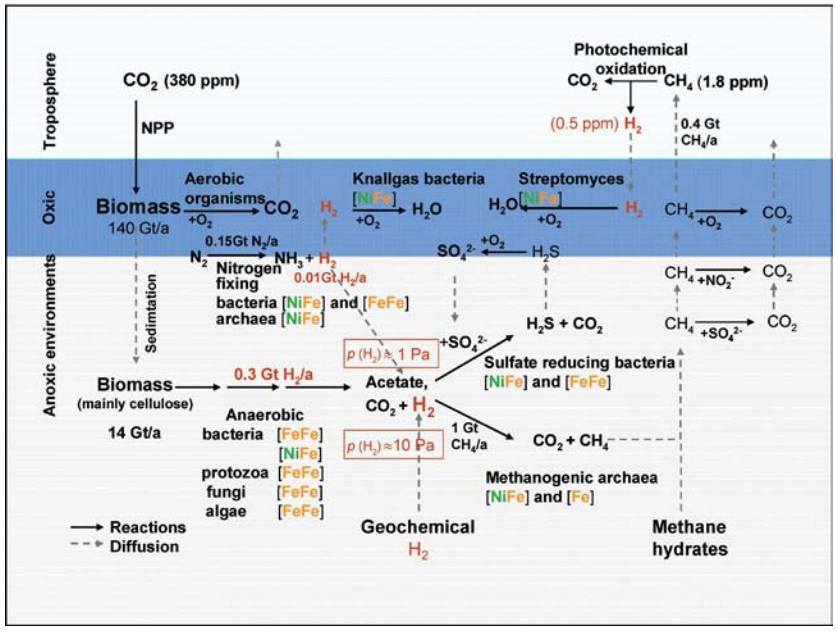

Figure 1. Reactions and organisms involved in the global H_2 cycle. The three types of hydrogenases involved are abbreviated as [NiFe], [FeFe], and [Fe]. NPP stands for net primary production by oxygenic photosynthesis.

[a] Max Planck Institute for Terrestrial Microbiology, Karl-von-Frisch-Strasse 10, 35043 Marburg, Germany

redox potential range in which protons are reduced to H_2 and H_2 is oxidized by microorganism in the H_2 cycle and in which hydrogenases catalyzing these reactions and sensing H_2 physiologically must function.

H_2 is not only formed by anaerobic organisms involved in biomass fermentation to acetate, CO_2, and H_2 but also as side product of nitrogen fixation: per mol N_2 reduced to 2 NH_3, at least 1 H_2 is regenerated.[6] Estimates are that approximately 0.15 Gt N_2 are fixed by microorganisms annually,[7] leading to the formation of approximately 0.01 Gt H_2 yearly. Nitrogen fixers can be anaerobes or microaerophiles. Thus, part of the H_2 generated as by product of N_2 fixation is generated where O_2 is available as electron acceptor. Knallgas bacteria are capable of using this H_2 together with O_2 as energy source.

A third source of H_2 in the H_2 cycle is atmospheric methane. Approximately 0.4 Gt CH_4 escape annually from the anoxic environments into the atmosphere and is oxidized there photochemically with O_2 to CO_2 with H_2 as a side product. The major sinks for atmospheric H_2 are aerobic soils, which must therefore harbor microorganisms that can oxidize H_2 at H_2 partial pressures below 0.1 Pa because the atmospheric concentrations of H_2 are in the order of 0.5 ppm.[8] These microorganisms have recently been identified to be *Streptomyces* species.[9]

Three types of hydrogenase are presently known to be involved in the hydrogen cycle, [NiFe]-hydrogenases, [FeFe]-hydrogenases, and [Fe]-hydrogenase,[4] the active site structures of which are depicted in Figure 2. Interestingly, the three hydrogenases have in common a low-spin iron(II) with CO and cyanide ligands (the acyl group in [Fe]-hydrogenase is considered to be functionally equivalent to cyanide). They differ, however, significantly in O_2 tolerance. Whereas [FeFe]-hydrogenases function only under strictly anaerobic conditions, [NiFe]-hydrogenases and [Fe]-hydrogenase can also be active in the presence of O_2.[10,11] Another difference appears to be that [FeFe]-hydrogenases preferentially function in the direction of H_2 formation and [NiFe]-hydrogenases preferably in H_2 uptake, reflecting the fact that [FeFe]-hydrogenases generally use electron acceptors/donors with a more negative redox potential than those used by [NiFe]-hydrogenases. But there are exceptions. Cytochrome c_3 reduction with H_2 in *Desulfovibrio vulgaris* is catalyzed by [FeFe]-hydrogenase when the sulfate reducer grows on media deprived of nickel that is required for the synthesis of its [NiFe]-hydrogenases (Figure 1). Moreover, the reduction of protons with electrons coming from formate ($E_o' = -430$ mV) in *E. coli*, which can also grow aerobically, is catalyzed by a [NiFe]-hydrogenase. Since archaea can only synthesize [NiFe]-hydrogenases, they must use these enzymes for both H_2 formation and uptake.

In addition to the biological function of hydrogenases, there are presently four main areas of hydrogenase research, which are individually addressed in the current volume of the *European Journal of Inorganic Chemistry*:

(1) Mechanism: The pK of H_2 has been estimated to be 35. Thus, heterolytic cleavage of H_2 in H_2O at pH 7 is endergonic by approximately 160 kJ/mol. This activation energy must be overcome by the enzymes catalyzing H_2 oxidation. From the structures and spectroscopic properties determined to date, it is evident that in all three types of hydrogenases a low-spin iron(II) carbonyl plays an important role in H_2 activation.[12,13] With small molecule mimics of hydrogenases it is hoped to get a better insight in to their mechanism of action.[14]

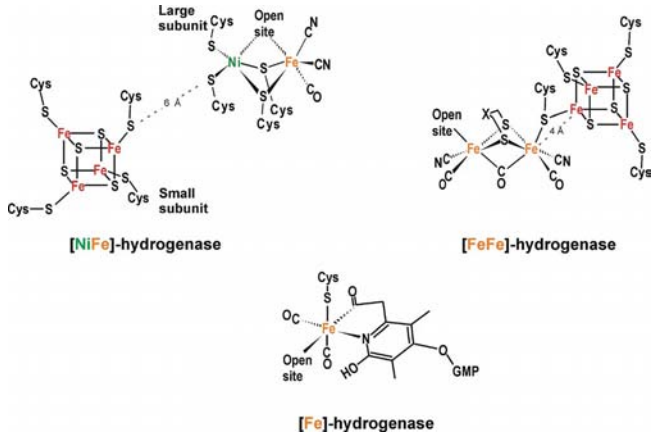

Figure 2. The metal sites of the three types of hydrogenases involved in the H_2 cycle (see Figure 1). GMP denotes guanylyl residue. Reproduced from ref.[4]

(2) Biosynthesis: Although the active sites of the three hydrogenases are structurally related, they are not so evolutionary. The biosynthesis of their prosthetic groups has mechanistically nothing in common. The intrinsic cyanide ligands of [NiFe]-hydrogenase starts from carbamoyl phosphate[15] whereas that of [FeFe]-hydrogenases starts from tyrosine.[16] Also the intrinsic CO ligands in the three types of hydrogenases are of different origin, although at present only the origin of the CO in [FeFe]-hydrogenases from tyrosine is really known.[17,18]

(3) Evolution: A current hypothesis is that pre-biotic chemistry started out with H_2 and CO_2, with iron and nickel catalysts and iron–sulfur clusters as electron carriers.[19–21] Indeed, one of the phylogenetically deepest rooted microorganisms, *Methanopyrus kandleri*, grows at a temperature near 100 °C on H_2 and CO_2 using three different types of [NiFe]–iron–sulfur proteins for H_2 activation. If the "iron–sulfur world" hypothesis[19] is correct, then NiFe/FeS centers must exist that can catalyze the activation of H_2 outside proteins. In turn, the discovery of NiFe/FeS or of FeFe/FeS complexes that can catalyze the reversible oxidation of H_2 will contribute to our understanding of prebiotic evolution.

(4) Application: Currently, platinum is used to activate H_2 and O_2 in fuel cells with H_2 as electron donor and O_2 as electron acceptor. Platinum is not only expensive but also only available in limited amounts. The discovery of a stable Ni/Fe-based catalyst for H_2 activation would therefore be a great step forward. To learn from hydrogenases may be a way to get there. Bio-inspired catalysts could be used in technical energy conversions. But to use hydrogenases themselves is probably a fiction, since enzymes are too la-

bile and relatively too expensive to be employed in processes that do not significantly increase the value of the product as is the case in energy conversions.

Acknowledgments

I thank Dr. Seigo Shima from our Institute for helpful discussions.

[1] R. K. Thauer, E. Stackebrandt, W. A. Hamilton, *Sulphate-Reducing Bacteria* (Eds.: L. L. Barton, W. A. Hamilton), Cambridge University Press, **2007**, pp. 1–37.
[2] R. K. Thauer, A. K. Kaster, H. Seedorf, W. Buckel, R. Hedderich, *Nat. Rev. Microbiol.* **2008**, *6*, 579–591.
[3] R. Conrad, *Environ. Microbiol. Rep.* **2009**, *1*, 285–292.
[4] R. K. Thauer, A. K. Kaster, M. Goenrich, M. Schick, T. Hiromoto, S. Shima, *Annu. Rev. Biochem.* **2010**, *79*, 507–536.
[5] R. K. Thauer, K. Jungermann, K. Decker, *Bacteriol. Rev.* **1977**, *41*, 100–180.
[6] J. Christiansen, D. R. Dean, L. C. Seefeldt, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **2001**, *52*, 269–295.
[7] M. B. Peoples, D. F. Herridge, J. K. Ladha, *Plant Soil* **1995**, *174*, 3–28.
[8] R. B. Guo, R. Conrad, *Soil Biol. Biochem.* **2008**, *40*, 1149–1154.
[9] P. Constant, S. P. Chowdhury, J. Pratscher, R. Conrad, *Environ. Microbiol.* **2010**, *12*, 821–829.
[10] J. A. Cracknell, A. F. Wait, O. Lenz, B. Friedrich, F. A. Armstrong, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 20681–20686.
[11] O. Lenz, M. Ludwig, T. Schubert, I. Burstel, S. Ganskow, T. Goris, A. Schwarze, B. Friedrich, *Chemphyschem* **2010**, *11*, 1107–1119.
[12] A. Silakov, C. Kamp, E. Reijerse, T. Happe, W. Lubitz, *Biochemistry* **2009**, *48*, 7780–7786.
[13] M. E. Pandelia, H. Ogata, W. Lubitz, *Chemphyschem* **2010**, *11*, 1127–1140.
[14] F. Gloaguen, T. B. Rauchfuss, *Chem. Soc. Rev.* **2009**, *38*, 100–108.
[15] A. Bock, P. W. King, M. Blokesch, M. C. Posewitz, *Adv. Microb. Physiol.* **2006**, *51*, 1–71.
[16] R. C. Driesener, M. R. Challand, S. E. McGlynn, E. M. Shepard, E. S. Boyd, J. B. Broderick, J. W. Peters, P. L. Roach, *Angew. Chem.* **2010**, *122*, 1731; *Angew. Chem. Int. Ed.* **2010**, *49*, 1687–1690.
[17] L. Forzi, P. Hellwig, R. K. Thauer, R. G. Sawers, *FEBS Lett.* **2007**, *581*, 3317–3321.
[18] E. M. Shepard, B. R. Duffus, S. J. George, S. E. McGlynn, M. R. Challand, K. D. Swanson, P. L. Roach, S. P. Cramer, J. W. Peters, J. B. Broderick, *J. Am. Chem. Soc.* **2010**, *132*, 9247–9249.
[19] G. Wachtershauser, *Chem. Biodiversity* **2007**, *4*, 584–602.
[20] S. E. McGlynn, D. W. Mulder, E. M. Shepard, J. B. Broderick, J. W. Peters, *Dalton Trans.* **2009**, 4274–4285.
[21] C. Huber, W. Eisenreich, G. Wachtershauser, *Tetrahedron Lett.* **2010**, *51*, 1069–1071.

Received: November 30, 2010
Published Online: February 10, 2011

