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Hydrogenases for biological hydrogen production

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ABSTRACT

Biological H_2 production offers distinctive advantages for environmental protection over existing physico-chemical methods. This study focuses specifically on hydrogenases, a class of enzymes that serves to effectively catalyze H_2 formation from protons or oxidation to protons. It reviews the classification schemes (i.e. [NiFe]-, [FeFe]-, and [Fe]-hydrogenases) and properties of these enzymes, which are essential to understand the mechanisms for H_2 production, the control of cell metabolism, and subsequent increases in H_2 production. There are five kinds of biological hydrogen production methods, categorized based upon the light energy requirement, and feedstock sources. The genetic engineering work on hydrogenase to enhance H_2 production is reviewed here. Further discussions in this study include nitrogenase, an enzyme that normally catalyzes the reduction of N_2 to ammonia but is also able to produce H_2 under photo-heterotrophic conditions, as well as other applicable fields of hydrogenase other than H_2 production.

1. Introduction

Growing concerns with severe global warming and fossil fuel depletion have prompted extensive research in pursuit of alternative and clean energy sources. Among the various candidates, hydrogen gas (H_2) is regarded as the most promising future energy carrier as it has higher energy content by 2.75 times compared to hydrocarbon fuels (gasoline) and produces only water upon combustion. In addition, H_2 can be directly used in a fuel cell, generating electricity with high efficiency (Momirlan and Veziroglu, 2002). The pioneering notion of a "Hydrogen Energy System" drew inspiration from the French science-fiction novel "The Mysterious Island" by Jules Verne (1874), where the idea of using H_2 as an energy carrier first appeared (Mason, 2007). To date however, over 90% of the production of H_2 remains based upon steam reforming of hydrocarbons and coal gasification, which starts from fossil fuels and requires high temperature and pressure conditions. Meanwhile, biological methods have great potential for the production of H_2 in an environmentally friendly way.

H_2 is the most common element in the universe, and it is expected that the Earth's early atmosphere was reducing one predominated by H_2. At present, the atmosphere has turned to an oxidizing one, which was carried out mostly by biological processes that still continue (Vignais and Billoud, 2007). Many bacteria obtain energy by the oxidation of H_2 assisted by a number of complex mechanisms, and O_2 is released by the oxidation of water via photosynthesis. Meanwhile through a less well appreciated process, various species evolve H_2 under anaerobic conditions. Actually, this is a proximate and everyday process for individuals: the bacteria in our digestive tract produce H_2 (Cammack et al., 2001). However, most of this produced H_2 is undetectable, because it is immediately recycled by other bacteria.

Life depends on numerous series of chemical reactions, yet many of these reactions proceed too slowly on their own. Hence, nature has designed catalysts to greatly accelerate the rates of biochemical reactions, which we now refer to as enzymes (Copeland, 2000). The key enzyme involved in catalyzing H_2 formation from protons or oxidation to protons is hydrogenase. The reaction ($2H + 2e^- \leftrightarrow H_2$) is reversible, and its direction depends on the redox potential of the components that are able to interact with hydrogenase (Vignais and Colbeau, 2004). In addition, nitrogenase, an enzyme that normally catalyzes the reduction of N_2 to ammonia, is able to reduce protons to H_2 as a byproduct under photo-heterotrophic conditions (McKinlay and Harwood, 2010).

A study of hydrogenase is essential for understanding the H_2 production mechanism, controlling cell metabolism, and finally increasing H_2 production. Since 1931, when hydrogenase was first described by Stephenson and Stickland, extensive research has been conducted in this area (Mertens and Liese, 2004). The sequences of ca. 450 hydrogenases are now available, and they are well categorized by their specific characteristics and active site models have been developed (Meyer, 2007).

In the present work, various aspects of hydrogenases (classification and properties), biological H_2 production (BHP) systems, and genetic engineering work on hydrogenase for enhanced H_2 production are reviewed.

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2. Classification and properties of hydrogenase

As categorized in Fig. 1, various hydrogenases are known today, and all but one are involved directly or indirectly in energy metabolism either catalyzing H_2 oxidation (H_2 consumption) or H^+ reduction (H_2 evolution). One family of hydrogenase serves as H_2-sensing components in the genetic regulation of hydrogenase expression, as found in several autotrophic Proteobacteria, e.g., *Ralstonia eutropha*, *Rhodobacter capsulatus*, and *Bradyhizobium legyminosarum* (Kleihues et al., 2000). Detailed investigation of sequences of various hydrogenases have confirmed that despite their diversity in many respects (size, structure, electron donors, etc.), hydrogenases could be split into three distinct classes, [NiFe]-, [FeFe]-, and [Fe]-hydrogenase depending on the metal atoms on their active sites (Meyer, 2007).

2.1. [NiFe]-hydrogenase

[NiFe]-hydrogenases constitute the largest number of hydrogenases. It is considered that Ni-containing hydrogenases tend to be less sensitive than [FeFe]-hydrogenases to the inhibition by CO and O_2 (Cammack et al., 2001). The core enzyme consists of a α, β heterodimer; the α-subunit being larger one and contains biometallic active site, whereas the small β-subunit possesses Fe–S clusters, which transfer electrons between the active site and electron accepter/donor binding site. As shown in Fig. 2a, a bimetallic NiFe center is coordinated with the S-atoms of 4 cystein residues. Also, non-proteinous ligands, one CO and two CN are coordinated with Fe atom (Happe et al., 1997). A full sequence analysis of the small and large subunits led to the classification of [NiFe]-hydrogenases into four groups, which is consistent with the functions of the enzymes.

Group I includes uptake [NiFe]-hydrogenases, which are generally found in *Wolinella succinogenes*, *Aquifex aeolicus*, *Thiocapsa roseopersicina*, and some *Desulfovibrio* sp. These enzymes are membrane-bound, which link the oxidation of H_2 to the reduction of anaerobic electron acceptors, such as NO_3^-, SO_4^{2-}, furmarate, or CO_2 (anaerobic respiration) or to O_2 (aerobic respiration), with the recovery of energy in the form of a proton-motive force. In terms of the structure, these enzymes are characterized by the presence of a long signal peptide at the N terminus of their small subunit (Sargent et al., 2006). The function of a signal peptide is to serve as signal recognition targeting the fully folded heterodimer to the membrane and the periplasm.

Group II includes (a) cytoplasmic H_2 sensors and (b) cyanobacterial uptake [NiFe]-hydrogenases, whose small subunit does not contain a signal peptide at its N terminus. Group II(a) enzymes remain in the cytoplasm, and their role is to detect the presence of H_2 in the environment and to trigger a cascade of cellular reactions controlling the synthesis of hydrogenases (Kleihues et al., 2000).

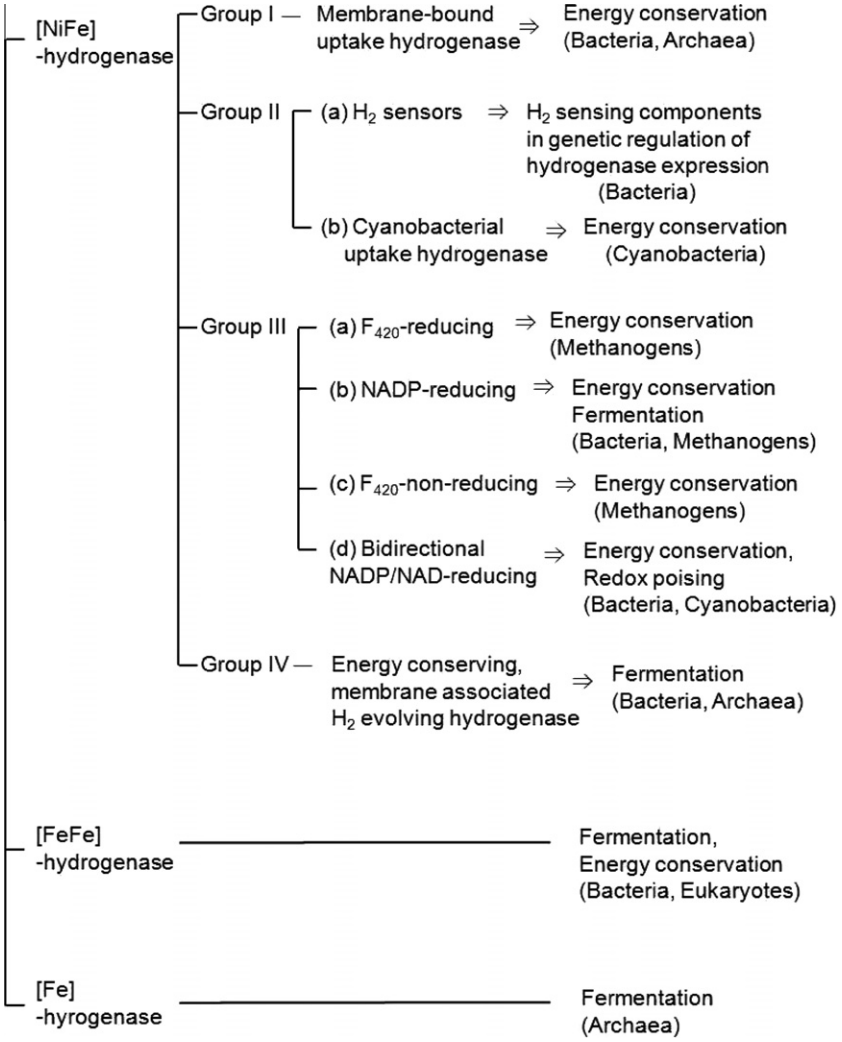

Fig. 1. Classification of hydrogenases (Modified from Cammack et al., 2001, and Vignais and Billoud, 2007).