A Review on Hydrogenases and Their Structures and Mechanisms

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ABSTRACT: Hydrogenases is a group of enzyme that catalyze the formation or consumption of hydrogen atom. In the nature, there are 3 categories of hydrogenases, each of which has unique function. [Ni-Fe] hydrogenase catalyze H_2 binding and oxidation. [Fe-Fe] hydrogenases has a high affinity in H_2 production. [Fe] hydrogenases selectively catalyzes hydride transfer. There are also hydrogenase contained other transition metal center only, such as Mn hydrogenases, which is completely man-made. This article mainly reviews 3 kinds of natural hydrogenase and a Mn hydrogenase. A recent progress of [Ni-Fe] hydrogenase and [Fe-Fe] hydrogenase is included as well. Specifically, an conformation change in [Ni-Fe] hydrogenase will be illustrated carefully too. In general, the essay demonstrate and structure and function of some representative hydrogenase system to help readers understand some basic principles of hydrogenases

Introduction

Hydrogenases, abbreviated as H_2 ases, is referred to a kind of enzyme that catalyze formation and usages of hydrogen in creatures including prokaryotic and eukaryotic creatures. It is widely participated in the oxidation of H_2 and the reverse process, which is reduction of proton to hydrogen. The oxidative procedure generally includes hetero cleavage of hydrogen molecule while the reduction includes combination of proton and hydride ion to form hydrogen. Because of their ideal properties in hydrogen reversible transformation, many researchers thinks that H_2 ases may be an excellent material for hydrogen storage. Up to now, researchers have developed some categories of hydrogenases that does not exist naturally by reconstructing active sites with a new transition metal, or simply with modification of coordination environment in its active site. Generally, naturally existed H_2 ases are divided into three categories, according to the transition metal includes inside. The three categories are [Ni-Fe] Hydrogenaes, [Fe-Fe] hydrogenases and [Fe] hydrogenases. Their main difference lays at the transition metal included in its active site and the reaction these enzymes catalyzes. This article reviews the prominent features of each hydrogenase type, with particular emphasis on structure, function, and mechanism that have aided in enzyme elucidation. For Ni-Fe hydrogenases and Fe hydrogenases, an recent progress is processed to demonstrates functions and mechanisms of these two types of hydrogenases specifically.

1. Ni-Fe Hydrogenases

Ni-Fe Hydrogenases is the most studied hydrogenases among these 3 categories and has a propensity towards catalyzing H_2 binding and oxidation. Usually the [Ni-Fe] Hydrogenases all has a [Ni-Fe] cluster wrapped inside the protein, and several Fe-S clusters as well. The distance between these clusters is within the maximum distance of the electron transmitting distance, which is close to 14Å, so that the electrons are able to conduct from the bare clusters to other clusters, eventually reaching the active sites. By cryo-EM, Li et all. has clarified the electron

transmission pathway of the Ni-Fe HydABCSL Hydrogenases. This hydrogenase consists of five subunits, namely, HydA, HydB, HydC, HydL, and HydS. The hydrogenase mainly catalyzes the oxidation of hydrogen to generate protons and reduces NAD to NADH. The reaction equation is as follows:

$$NADH + 2Fd_{red} + 3H^{+} \leftrightarrow$$
$$NAD + 2Fd_{ox} + 2H_2 .$$

According to the reaction formula, every two molecular hydrogen molecules transfers $4e^-$ each time, Two of them were transferred to NADH, and the other two reduced Fd_{ox} to Fd_{red}. The arrangement of its metal clusters presents a double "y" shape, with the active site Ni-Fe cluster located at the end of one "y". This arrangement of the metal clusters indicates the possibility of more than one electron transport pathway. One of the two electron transport pathways is B5-C1-B1-A1-A2-A4-A5. However, A5 is an inactive endpoint that cannot transfer electrons to the molecule outside the protein, so the electron transport trajectory is blocked. Another pathway is NiFe-S1-A3-A2-A1-B1-C1-B5 that transmits two electrons to NADH. The third pathway, via NiFe-S1-A3-A2-A1-B1-C1-B2-B3-B4, transmits 2 electrons to Fd, thus reducing Fd_{ox} to Fd_{red}. Notably, protein conformational conversion is also required for electrons from B1-C1-B2. When HydLC is rotated to a close connection site with HydLB, the conformation is known as bifurcation state. At this state, the electrons can move between B5-C1, but do not move freely between C1-B2. The opposite is true when rotation to the other conformation, known as electron transduction state. Such a mechanism allows the electrons to gradually transmit between different parts of the protein, avoiding the short circuit of the electron transport path, and then controlling the rate of biochemical reactions.

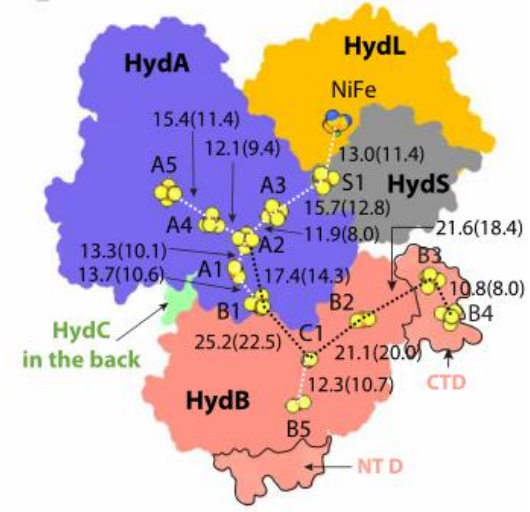

Figure 1. The structure and relative location of each cluster in Ni-Fe HydABCLS hydrogenase.

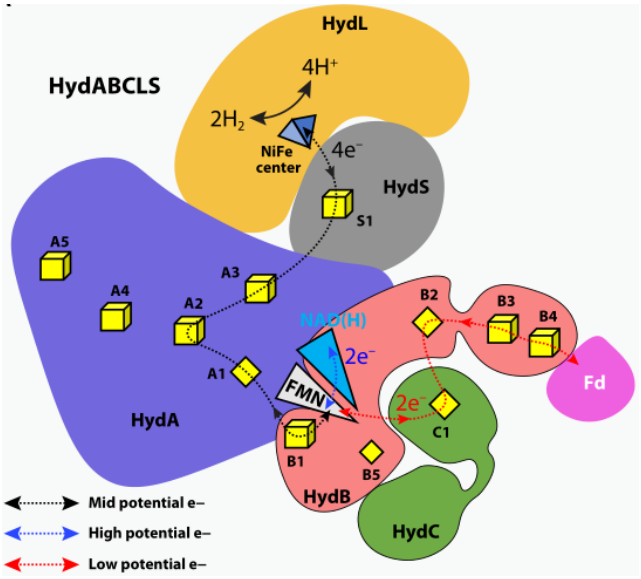

Figure 2. Two possible electron transferring pathway of HydABCLS.

2. Fe-Fe Hydrogenases

Found within anaerobic prokaryotes and lower eukaryotes, [FeFe] hydrogenases usually function as high-activity H_2-producing enzymes. Therefore research on [FeFe] hydrogenases may stimulate methods for releasing hydrogen gas in hydrogenic energy cells. Notably, this is the only type of hydrogenase found within eukaryotes and the enzyme is localized within organelles such as chloroplasts and hydrogenosomes. A [FeFe] hydrogenases is extracted from *Clostridium*

pasteurianum and its protein structure is resolved by high-resolution XRD, as Peters et al reported in the journal ***Science***. The molecule has the shape of a mushroom, which canopy contains 2 / 3 of the amino acids of the protein, and an active cluster HC buried inside. This metal cluster is composed of 4Fe-4S and a 2Fe-2S cluster. The 2Fe-2S cluster is similar to the shape of a ship, with regular octahedron-configuration iron atoms at the front and stern, and bridge-connected sulfur atoms in the hull depression. The two sulfur atoms and two iron atoms are also connected by a bridging CO ligand. There are also 34Fe-4S cluster and a 2Fe-2S cluste below. FS4C and FS2 cluster are directly exposed to the surface of the protein, and might be sites that receive or give electrons, according to the authors.

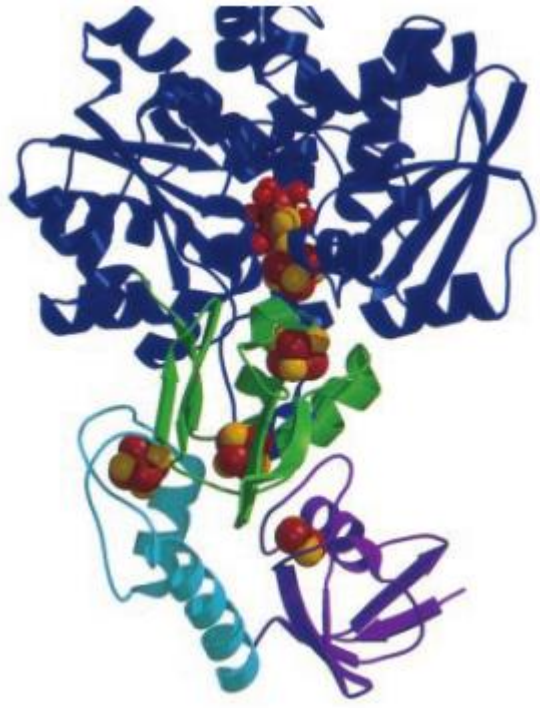

Figure 3. The 3D structure of the Fe-Fe hydrogenase extracted from *Clostridium pasteurianum*

3. Fe hydrogenases and hydrogenases with other transition metals.

The [Fe] hydrogenases are found exclusively in methanogenic archaea and are upregulated under Ni-limited conditions. Only a few examples of [Fe] hydrogenases have been characterized. [Fe] hydrogenases mainly catalyzes transfer of H^- ion and hydrogenates cationic site of compound. Its structure contains Fe atoms but does not includes Fe-S clusters. Fe(II) are usually octahedrally coordinated to H-H, CO, Cys and other organic ligands.

Hydrogenases, enzymes that activate molecular H_2, exclusively utilize Ni and Fe in [NiFe]-, [FeFe]- and [Fe]- hydrogeanses. However, other transition metals are known to activate or catalyse the production of hydrogen in synthetic systems. Pan et all. constructed and synthesized a [Mn] hydrogenase, modeling after the structure of [Fe] hydrogenase. The active site of it contains only Mn as catalytic metal centre. This Mn complex is able to heterolytically cleave H_2 as well as catalyse hydrogenation reactions. The incorporation of the model into an apoenzyme of [Fe] hydrogenase results in a [Mn] hydrogenase with an enhanced occupancy-normalized activity over an analogous semi-synthetic [Fe] hydrogenase. It is a unprecedented attempt to design and synthesize a hydrogenase that does not exist naturally. The attempt offers the idea that with precise calculation and subtle design, we can develop hydrogenases with certain structure and function. These strategy may broaden the application of hydrogenase in reversible hydrogen storage and finally boost the development of hydrogen energy in the near future.

Reference

(1) Pan, H. J.; Huang, G.; Wodrich, M. D.; Tirani, F. F.; Ataka, K.; Shima, S.; Hu, X. A catalytically active [Mn]-hydrogenase incorporating a non-native metal cofactor. *Nat. Chem.* **2019** *11*, 669–675.

(2) Peters, J. W.; Lanzilotta, W. N. ; Lemon, B. J.; Seefeldt, L. C. X-ray Crystal Structure of the Fe-Only Hydrogenase (CpI) from Clostridium pasteurianum to 1.8

Angstrom Resolution. *Science* (Washington, D. C.) **1998**, *282*, 1853-1858.

(3) Feng, X.; Schut, G. J.; Haja, D. K.; Adams, M. W. W. , Li, H. Structure and electron transfer pathways of an electron-bifurcating NiFe-hydrogenase. *Science Advances* **2022**, *8*, 7546.

(4) Sickerman, N. S.; Hu, Y. *Metalloproteins*; Humana Press: New York, **2019**; pp 65-82