Chapter 23
Structure of Hydrogenase in Biohydrogen Production Anaerobic Bacteria

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ABSTRACT
Hydrogenase plays an important role in the process of biohydrogen production. Hydrogenases have very unique active sites and are classified into three groups according to the metal composition of the active sites: the [Ni-Fe] hydrogenase, [Fe-Fe] hydrogenase, and [Fe-only] hydrogenase. In this paper, the crystal structures and active sites of three kinds of hydrogenases are examined and compared. These enzymes have an unusual structural feature in common. Their similar active site indicates that the catalytic mechanism of hydrogen activation is probably similar. The understanding of the catalytic mechanisms for the three kinds of hydrogenases may help achieve the industrialization process of hydrogen energy production. Moreover, the future research direction about the hydrogenases from auto-aggregative bacteria and the chemical mimic of hydrogenases structure is discussed.

INTRODUCTION
The aim of this chapter is to review the research development of hydrogenase (Hase) in anaerobic bacteria and to provide reliable information regarding the structure of Hase in anaerobic bacteria. Toward this goal, the advancement in molecular structure discovery and active site studies of Hase in anaerobic bacteria is presented. Future research directions of Hase are then presented.

BACKGROUND
With the increasing demand for energy around the globe, the fossil fuel reserves are being depleted. In addition, the excessive use of fossil fuels is one of the primary causes for global climate change and air pollution. It is urgent to find alternative sources of energy to solve this issue (Zhu et al., 1999; Ren et al., 1997; Lay, 2000; Kataoka, 1997). Microorganisms have effective systems for hydrogen production by utilizing carbohydrate in pyruvate route of metabolic pathway. Due to
the pathway of carbohydrate metabolism and the bacterial hydrogen production, the specific rate of hydrogen conversion can reach to \(10\text{molH}_2/\text{mol-glucose}\) theoretically (Tanisho, 2000), which is a high level in the industrialization of hydrogen production. Hydrogen, as a clean energy source, is expected to be a substitute for fossil fuel in the 21st century, since it only generates water when combusted. With the sharp increase in energy requirements, hydrogen, as a clean fuel produced from bacteria, has been a much-pursued topic for researchers.

In some anaerobic microorganism, some proteins were identified in 1891 that decomposed formic acid to hydrogen and carbon dioxide, and were named as hydrogenases by Stephenson and Strickland (1931). Hydrogenase was first isolated and purified from *Clostridium pasteurianum* by Chen and Mortenson (1974). However, at that time, the discovery of hydrogenases didn’t attract too much research interest. Because of the abundance of oil and the lack of concern for climate change in those days, the benefits of low environmental pollution and potentials for sustainable development from hydrogen were not pursued.

Hydrogenases (Hase) are enzymes that catalyze the reversible oxidation process or production of molecular hydrogen. They can be found in a wide variety of organisms. Hydrogenase is the key terminal enzyme in electron delivery within biological hydrogen metabolism. There are three possible pathways of bacterial hydrogen production: pyruvate dehydrogenase pathway, pyruvate-formylrendase pathway, and NADH + H+ → H2 pathway (He et al., 2004; Tanisho, 2000; Gray & Gest, 1965). Hydrogenase catalyses the formation and decomposition of the simplest molecule, i.e., hydrogen. It plays a very important role in hydrogen metabolism and the energy production in bacteria (Nicolet, 2000; Tamagnini et al., 2002).

Over the past a few decades, researchers have paid much attention on the biochemical and biophysical properties of hydrogenases. Most of the studies focused on the structure and expression of gene, the stereo structure and the active center of hydrogenases, and the electron delivery mechanism and so on. Now, the structures and catalytic mechanisms of different hydrogenases from a myriad of microorganisms have been illustrated in varying degrees of detail. The study on the structures, functions and catalytic mechanisms of hydrogenases would provide an important theoretical basis for the research and application of biological hydrogen production. In recent years, more and more attention has been paid to identifying and developing new bacterial strains and modifying bacteria or hydrogenases through genetic engineering. Many promising results have shown the importance of studies on hydrogenases.

**STRUCTURE AND ACTIVE SITE OF HYDROGENASES**

In recent years, with the rapid development of biotechnology and protein identification technology, the studies of structures and functions of Hase have made great strides. One type of Hase, [Ni-Fe] Hase, which is comprised of a large subunit and a small subunit, was found in rod and vibrio shaped bacteria. The active center of [Ni-Fe] Hase is comprised of nonprotein diatomic ligands of the Fe atom, and there are two cyanide ligands (CN) and one carbon monoxide bridge (CO) bound to the Fe atom. The active site is located in the large subunit, and there are [Fe-S] clusters in the small subunit (Volbeda et al., 2005). Another type of Hase, [Fe-Fe] Hase, was found in bacteria and eukaryotes. The X-ray crystallographic structures and the spectroscopic data have been reported by Peters et al. (1998). The [H] cluster, the active site at which protons are reduced to dihydrogen (or dihydrogen oxidised to protons) can be viewed as a conventional [4Fe-4S]-cluster which is linked to a [2Fe-3S]-subsite by a protein backbone bridging cysteinyl sulfur ligand. At the subsite, a terminal carbon monoxide, a bridging carbon monoxide and a cyanide ligand are bound at each iron atom that
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