Hydrogenase structure, function, and expression

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During the past decade we have made significant contributions to the understanding of the molecular architecture and mechanism of Fe-only hydrogenases. In nature hydrogenases occur mainly in microorganisms where they either couple the oxidation of molecular hydrogen to provide reducing equivalents for energy yielding processes or in proton reduction where protons serve as an electron acceptor for reducing equivalents that accumulate during microbial fermentation. Our structural work has revealed that the catalytic site of these enzymes exist as a complex bridged metal assembly in which a [4Fe-4S] cluster is covalently linked to a 2Fe containing subcluster via a bridging Cys thiolate. The 2Fe subcluster is a uniquely organometallic biologically derived prosthetic group with both carbon monoxide and cyanide ligands to Fe.

Some of our more recent work concerning these enzymes involves generating a suitable heterologous expression system. Generating such an expression system is not trivial since the biosynthesis and maturation of an active Fe-only hydrogenase requires a number of other gene products in addition to the enzyme structural genes. Recently, the research groups of Ghirardi and Seibert at the National Renewable Energy Laboratory have implicated the involvement of several gene products in the maturation of the Fe-only hydrogenase and have reported the successful heterologous expression of the Fe-only hydrogenase from the green algae *Chlamydomonas reinhardtii* and have demonstrated that active hydrogenase can be expressed in *E. coli*. In some organisms the aforementioned gene products appear to be organized in a defined operon with the structural genes for the hydrogenase. The metabolically versatile bacterium *Shewanella oneidensis* possesses one such operon which consists of the structural genes for the large and small subunit of the enzyme and five additional apparent genes. Cloning the intact operon into *E. coli* expression vectors allows the heterologous expression of the hydrogenase evidenced by the detection of hydrogen evolution activities in *E. coli* extracts from cells in which controlled expression has been induced. We are currently optimizing expression of soluble active enzyme. The expression system will be utilized to generate hydrogenase site-specific amino acid substituted variants that will allow the role of individual amino acid residues in catalysis to be probed. In addition, defined deletions can be introduced into the accessory genes to gain insights into the mechanism of cluster synthesis and enzyme maturation.