BES006 Hyperthermophilic Multiprotein Complexes and Pathways for Energy Conservation and Catalysis

Principal Investigator: Michael W.W. Adams
Department of Biochemistry and Molecular Biology
University of Georgia
Athens, GA 30602
Phone: (706) 542-2060
Email: adams@bmb.uga.edu

DOE Program Manager: Dr. Robert J. Stack
Phone: (301) 903-5652
Email: robert.stack@science.doe.gov

Objectives

We are investigating the mechanisms of assembly of energy transducing systems, the processes that regulate energy-relevant chemical reactions, the architecture of biopolymers, and the active site protein chemistry leading to efficient bio-inspired catalysts. The novel protein complexes under study have the remarkable property of being synthesized (self-assembling) at temperatures near 100°C in a so-called hyperthermophilic microorganism. Moreover, the novel complexes are involved in the conversion of low potential reducing equivalents into gaseous end products such as hydrogen and hydrogen sulfide, or the oxidation of C1 compounds such as formate and CO, with the concomitant conservation of energy in the form of ion gradients. This is particularly relevant to the DOE mission since a fundamental problem in all photosynthetic reaction systems is the conversion of low potential reductant to a useable form of energy such as an ion motive force.

Technical Barriers

The model microbial system that is being used to study the energy conservation complexes is *Pyrococcus furiosus*. This archaeon grows optimally at 100°C and is also a strict anaerobe that grows in the absence of oxygen.

Abstract

P. furiosus obtains carbon and energy for growth by fermenting carbohydrates and producing H_2 and by reducing elemental sulfur (S°) to H_2S. It has a respiratory metabolism in which it couples H_2 production by a ferredoxin-dependent, membrane-bound hydrogenase (MBH) to ion translocation and formation of a membrane potential that *P. furiosus* utilizes to synthesize ATP. *P. furiosus* also contains a cytoplasmic hydrogenase (SHI) that has the rare property of evolving H_2 from NADPH, a reaction of utility in H_2 production systems. Addition of S° to *P. furiosus* prevents the synthesis of MBH and SHI, and induces the synthesis of a highly homologous membrane complex which we term MBX. MBX is proposed to oxidize ferredoxin, reduce S° and conserve energy by an as yet unknown mechanism. The specific aims of this research are: 1) to characterize the novel energy-conserving complex MBH, 2) to characterize the novel energy-conserving complex MBX, and 3) to structurally characterize native SHI and minimal forms of SHI and MBH. We are taking advantage of our recent development of a genetic system in *P. furiosus* to generate strains containing deletions of key genes, affinity-tagged enzymes and/or over-expressed forms of the enzymes of interest. We are also heterologously expressing in *P. furiosus* H_2-evolving membrane complexes from related archaea that are analogous to MBH. The results of this research will provide a fundamental understanding of energy conservation in *P. furiosus* that involve the metabolism of H_2 gas.

Progress Report

1. Engineering P. furiosus to overproduce its cytoplasmic [NiFe] hydrogenase SHI. SHI is a complex heterotetrameric enzyme that contains flavin and multiple iron-sulfur clusters (Figure 1). Using the new genetic system [1] we have generated a strain of *P. furiosus* that yields approximately10-

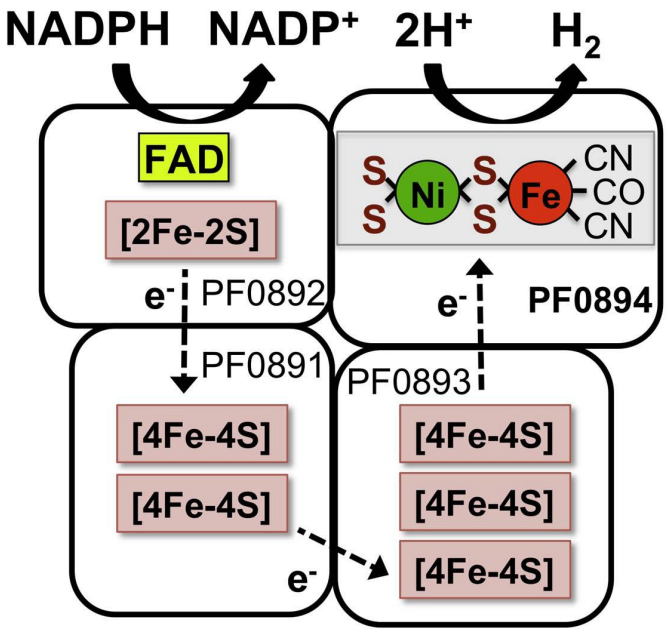

FIGURE 1. Proposed structure and cofactor content of hydrogenase I (SHI).

fold higher amounts of SHI compared to the native purification and produces the affinity-tagged enzyme containing a Step tag to facilitate purification [2].

2. Obtaining deletion strains of P. furiosus lacking both soluble hydrogenases or lacking the membrane-bound hydrogenase and construction of enzyme variants. A deletion mutant lacking both hydrogenases SHI and SHII has been obtained and this shows no phenotype [3]. This means that these enzymes are not essential for growth and we can, therefore, generate a range of 'minimal' and other variants of both enzymes. In addition, a deletion mutant of MBH was obtained and this strain, as expected, only grows in the presence of sulfur [3].

3. Evidence for the membrane-bound hydrogenase of P. furiosus as an ancestral H_2-evolving ion-pumping complex. The 14-subunit membrane-bound [NiFe] hydrogenase (MBH) of *P. furiosus* links the thermodynamically favorable oxidation of ferredoxin with the formation of hydrogen and conserves energy in the form of an ion gradient thereby representing a simple respiratory system within a single complex (Figure 2). This hydrogenase shows a modular composition represented by a Na^+/H^+ antiporter domain (Mrp) and a [NiFe] hydrogenase domain (Mbh). With the availability of a large number of microbial genome sequences, we have shown that homologs of Mbh and Mrp are ubiquitous in the microbial world and some species contain additional domains that catalyze the oxidation of formate, CO or NAD(P)H. The respiratory-type MBH of *P. furiosus* appears to be closely related to the common ancestor of complex I and [NiFe]-hydrogenases in general [4].

4. Purification of the Intact Functional Fourteen-Subunit Respiratory Membrane Bound [NiFe]-Hydrogenase Complex (MBH) of P. furiosus. MBH is encoded by a 14-gene operon with both hydrogenase and Na+/H+ antiporter modules. A His-tagged form MBH with the tag at the N-subunit (Figure 2) was expressed in *P. furiosus* and the detergent-solubilized complex purified under anaerobic conditions by affinity chromatography (unpublished data). Purified MBH contained all 14 subunits by electrophoretic analysis (13 subunits were also identified by mass spectrometry) and had a measured Fe:Ni ratio of 15:1, resembling the predicted value of 13:1. The as-purified enzyme exhibited a rhombic EPR signal characteristic of the ready Ni-B state. The purified and membrane bound forms of MBH both preferentially evolved H_2 with the physiological donor (reduced ferredoxin) as well as with standard dyes. The O_2 sensitivities of the two forms were similar (half-lives of ~15 hr in air), but the purified enzyme was more thermolabile (half-lives at 90 degrees C of 1 hr and 25 hr, respectively). Structural analysis of purified MBH (with John A Tainer, Lawrence Berkeley National Laboratory) by small angle x-ray scattering (SAXS) indicated a Z-shaped structure with a mass of 310 kDa, resembling the predicted value (298 kDa). The SAXS analyses reinforce and extend the conserved sequence relationships of group 4 enzymes and Complex I (NADH quinone oxidoreductase). This is the first report on the properties of a solubilized form of an intact respiratory MBH complex that is proposed to evolve H_2 and pump Na+ ions.

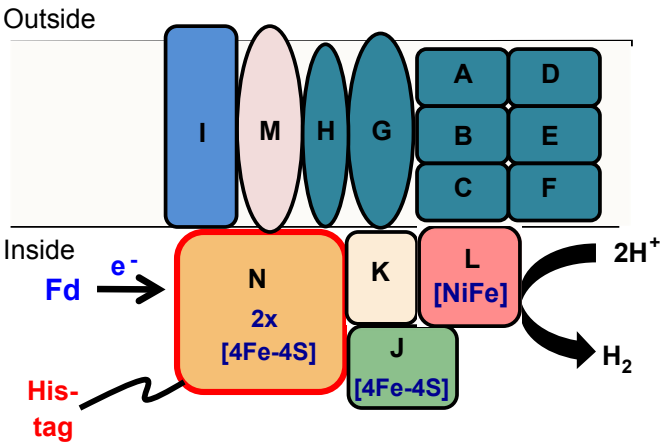

FIGURE 2. Proposed structure of the 14-subunit membrane-bound hydrogenase (MBH).

5. Engineering Hydrogen Gas Production from Formate in P. furiosus by Heterologous Production of an 18-Subunit Membrane-Bound Complex. Although H_2 gas has enormous potential as a source of reductant for the microbial production of biofuels, its low solubility and poor gas mass transfer rates are limiting factors. These limitations could be circumvented by engineering biofuel production in microorganisms that are also capable of generating H_2 from highly soluble chemicals such as formate, which can function as an electron donor. We have now engineered *P. furiosus,* which grows by fermenting sugars to produce H_2, has been engineered to also efficiently convert formate to H_2. Using a bacterial artificial chromosome vector, the 16.9 kb 18-gene cluster encoding the membrane-bound, respiratory formate hydrogen lyase (FHL) complex of *Thermococcus onnurineus* (Figure 3) was inserted into the *P. furiosus* chromosome and expressed as a functional unit [5]. This enabled *P. furiosus* to utilize formate as well as sugars as an H_2 source, and to do so at both 80° and 95°C, near the optimum growth temperature of the donor (*T. onnurineus*) and engineered host (*P. furiosus*), respectively. This accomplishment also demonstrates the versatility of *P. furiosus* for metabolic engineering purposes [5].

6. P. furiosus grows in the presence of oxygen. This organism had always been regarded as an obligate anaerobe that grows by fermenting carbohydrates to H_2, CO_2 and acetate. We have now shown [6] show that it is surprisingly tolerant to oxygen, growing well in the presence of 8% O_2 (v/v). Cell growth and acetate production were not affected by O_2 but H_2 production was reduced by 50%. Analysis of

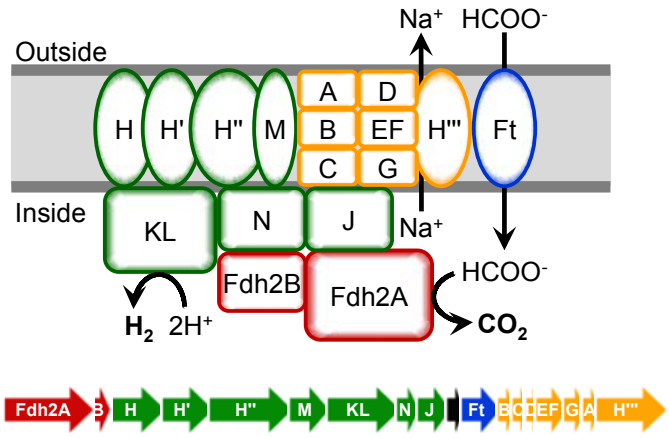

FIGURE 3. The formate hydrogen lyase (FHL) of *T. onnurineus* expressed in *P. furiosus* [5]. Colors represent the hydrogenase (green), formate dehydrogenase (red), the formate transporter (blue) and the Na^+/H^+ antiporter (yellow) modules of this multiprotein complex. The color-coded 18-gene operon that encodes FHL is also shown.

deletion mutants showed that electrons from fermentation are diverted to relieve O_2 stress at the level of reduced ferredoxin before H_2 production occurs. Superoxide reductase and flavo-diiron protein A were shown to play primary roles in removing O_2. These results bode well for using *P. furiosus* to obtain recombinant forms of O_2-requiring or utilizing enzymes [6].

7. Regulation of iron metabolism in P. furiosus. Iron is a key component of the enzymes of interest in *P. furiosus,* namely SHI, MBH and MBX, but how iron assimilation is regulated is completely unknown. Understanding this issue is important in order to ensure that highly up-regulated iron-containing enzymes are not limited for iron. In this work [7] we showed using DNA microarrays of deletion strains grown under iron-sufficient and -limiting conditions, in combination with *in vitro* DNA binding analyses, that DtxR is the key iron-responsive transcriptional regulator. DtxR regulates the expression of the genes encoding two putative iron transporters, Ftr1 and FeoAB while the expected regulator, Fur, was not functional [7].

Future Directions

In the future this research will focus on the characterization of solubilized MBH, the solubilization of the analogous MBX complex from *P. furiosus,* and on the characterization of analogous complexes from other hyperthermophilic archaea that are heterologously-expressed in *P. furiosus.*

Publication list acknowledging DOE grant FG05-95ER20175

1. Bridger, S.L, Lancaster, W.A., Poole, F.L., Schut, G.J. and Adams, M.W.W. Genome sequencing of a genetically-tractable *Pyrococcus furiosus* strain reveals a highly dynamic genome. *J. Bacteriol.* **2012,** *194,* 4097-4106

2. Chandrayan, S.K., McTernan, P.M., Hopkins, R.C., Sun, J., Jenney, F.E. and Adams, M.W.W. Engineering the hyperthermophilic archaeon *Pyrococcus furiosus* to overproduce its cytoplasmic [NiFe]-Hydrogenase. *J. Biol. Chem.* **2012,** *287,* 3257-3264

3. Schut, G.J., Nixon, W.J., Lipscomb, G.L., Scott, R.A. and Adams, M.W.W. Mutational analyses of the enzymes involved in the metabolism of hydrogen by the hyperthermophilic archaeon *Pyrococcus furiosus*. *Frontiers in Microbiology* **2012,** *3,* 163 (1-6)

4. Schut, G.J., Boyd, E.S., Peters, J.W and Adams, M.W.W. The modular respiratory complexes involved in hydrogen and sulfur metabolism by heterotrophic hyperthermophilic archaea and their evolutionary implications. *FEMS Microbiol. Rev.***2013,** *37,* 182-203

5. Lipscomb, G.L., Schut, G.J., Thorgersen, M.P., Nixon, W.J., Kelly, R.M. and Adams, M.W.W. Engineering hydrogen gas production from formate in a hyperthermophile by heterologous production of an 18-subunit membrane-bound complex. *J. Biol. Chem.* **2014,** *289,* 2873-2879

6. Thorgersen, M.P., Stirrett, K., Scott, R.A. and Adams, M.W.W. Mechanism of oxygen detoxification by the surprisingly oxygen-tolerant hyperthermophilic archaeon, *Pyrococcus furiosus. Proc. Natl. Acad. Sci. USA* **2012,** *109,* 18547-18552

7. Zhu, Y., Kumar, S., Menon, A.L., Scott, R.A. and Adams, M.W.W. (2013) "Regulation of Iron Metabolism by *Pyrococcus furiosus*" *J. Bacteriol.* **2014,** *195,* 2400-2407