Mutational Analysis of the Enzymes Involved in the Metabolism of Hydrogen by *Pyrococcus furiosus*

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Project Goals: The overall goal is to incorporate a systems-level approach to improve our understanding of microbial regulatory and metabolic networks related to hydrogen production through the development of new model organisms for microbial hydrogen production. We are using targeted approaches for the identification and characterization of enzymes and biochemical pathways relevant to biological hydrogen production. Advancing fundamental scientific knowledge in these areas are critical to characterizing enzymatic structure and function, modeling regulatory and metabolic networks, and engineering of enzymes and organisms to improve biological hydrogen production for a future hydrogen economy.

The hyperthermophilic archaeon *Pyrococcus furiosus* grows optimally near 100°C by fermenting carbohydrates to acetate, carbon dioxide and hydrogen gas (H_2). In the presence of elemental sulfur (S^0) it produces hydrogen sulfide rather than H_2. *P. furiosus* contains three distinct hydrogenases, which are enzymes that metabolize H_2. Two are cytosolic and are termed SHI and SHII. Both use NADP(H) as an electron carrier and each is encoded by a 4-gene operon. The third hydrogenase, MBH, is membrane-bound and uses the redox protein ferredoxin as an electron carrier. It is encoded by a 14-gene operon. In a previous study (1) we constructed deletion strains lacking the operons encoding SHI or SHII or both and showed that they exhibited no obvious phenotype under the usual growth condition (at 98°C using maltose as the carbon source). This study has now been extended to include biochemical analyses and growth studies under a variety of conditions using the ΔSHI and ΔSHII deletion strains together with strains lacking a functional MBH or more specifically, lacking the catalytic subunit (MbhL) of MBH. While deletion of either one or both cytosolic hydrogenases does not produce any obvious growth phenotype, the levels of hydrogenase activity in the cytoplasmic extracts are not affected in the ΔSHII strain but are strongly reduced in the ΔSHI strain (<10% compared to the parental strains). In the SHI and SHII double deletion strain, the cytosolic hydrogenase activity was below the detection limit. These data indicate that SHI is responsible for most of the hydrogenase activity in the cytoplasm. In contrast, the strain lacking the membrane-bound hydrogenase catalytic subunit showed no growth in the absence of S^0. This confirms the hypothesis that, in the absence of S^0, MBH is the enzyme that produces H_2. Moreover, these results show that in the absence of S^0 *P. furiosus* can only dispose of reductant generated from sugar oxidation in the form of H_2 gas. The deletion strain devoid of all three hydrogenases (ΔSHI ΔSHII Δ*mbhL*) grows only in the presence of S^0 and did not produce any detectable H_2. When the hydrogenase deletion strains and their parental strains were grown in the presence of limiting S^0 (0.5 g/L vs 2 g/L), both S^0 is reduced and H_2 is produced in the parental strains (ca. 50% H_2

produced compared to no added S^0). Interestingly, in Δ*mbhL*, a significant amount of H_2 is produced (ca. 20% compared to the parental strains) and growth was compromised (ca. 50% lower cell yield as compared to the parental strains). Therefore, the H_2 produced in Δ*mbhL* must be catalyzed by SHI, showing that this cytosolic 'uptake' hydrogenase can also produce H_2 from NADPH *in vivo*, even though this reaction is thermodynamically unfavorable. However, SHI cannot compensate for the absence of MBH since it does not enable significant growth of the Δ*mbhL* strain. We propose that the *in vivo* function of SHI is to recycle H_2 and provide a link between external H_2 and the intracellular pool of NADPH needed for biosynthesis. *P. furiosus* only uses a low potential ferredoxin in its glycolytic pathway, which is linked to MBH for the disposal of all reducing equivalents as H_2, generating an ion gradient for ATP generation in the process. The ability to recycle H_2 might have a distinct energetic advantage in the environment, but it is clearly not required for heterotrophic growth of the organism under the usual laboratory conditions.

Reference

1. Lipscomb, G. L., Stirrett, K., Schut, G. J., Yang, F., Jenney, F. E, Jr., Scott, R. A., Adams, M. W. W. and Westpheling, J. (2011) Natural competence in the hyperthermophilic archaeon *Pyrococcus furiosus* facilitates genetic manipulation: use for constructing multiple markerless deletions of genes encoding two cytoplasmic hydrogenases. *Appl. Environ. Microbiol.* **77,** 2232-2238

This work was supported by grants from the Office of Biological and Environmental Research (DE-FG02-08ER64690) and the Chemical Sciences, Geosciences and Biosciences Division (DE-FG05-95ER20175), Office of Basic Energy Sciences, Office of Science, U.S. Department of Energy.