Prospects for Hydrogen Production from Formate by *Methanococcus maripaludis*

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Objectives

We seek to understand the following features of hydrogenotrophic methanogenesis, all of which relate to strategies for the production of hydrogen from formate: First, for methanogens that use formate, how does formate feed electrons into the methanogenic pathway? Second, what is the basis for the uncoupling of methanogenesis from growth that is observed in hydrogenotrophic methanogens? Third, nitrogenase can be used to produce hydrogen; how is its activity regulated? Fourth, re-utilization of hydrogen must be prevented; what are the metabolic pathways be which hydrogen is utilized?

Technical Barriers

First, we must learn how to sustain growth, methanogenesis, and hydrogen production on formate in bioreactors. Second, we need to optimize the uncoupling of metabolism from growth. Third, we need to maintain maximum nitrogenase activity. Fourth, hydrogenotrophic methanogens use hydrogen; we need to figure out how to prevent re-utilization of hydrogen that is produced.

Abstract

Most methanogenic Archaea are hydrogenotrophic, specializing in the use of hydrogen to reduce CO_2 to methane. Even though these organisms have multiple hydrogenases that *use* hydrogen, there is good potential to use them for the net *production* of hydrogen. This is because some species can use alternative electron donors, most notably formate, and because some species have nitrogenases that can produce large amounts of hydrogen. *Methanococcus maripaludis* is one such species. In addition, *M. maripaludis* is the premiere model for hydrogenotrophic methanogens, grows well under laboratory conditions, and can be manipulated using an extensive set of genetic tools. In past work we studied the mechanisms for regulation of nitrogenase at the transcriptional and enzyme levels, in the process discovering and characterizing two novel regulators and generating mutants that had constitutive nitrogenase activity (1, 2). In recent work on methanogenesis, we have focused on mechanisms of energy conservation and pathways of hydrogen and formate utilization. R. Thauer proposed in 2008 that energy conservation depends on electron bifurcation (3). The pathways of methanogenesis and electron bifurcation are illustrated in Figure 1. Electrons from hydrogen reduce the flavin of heterodisulfide reductase (Hdr), where electron flow then bifurcates, reducing heterodisulfide (exergonic) and coupling this to reduction of a ferredoxin (endergonic). Since the reduced ferredoxin may be used for the endergonic first step on methanogenesis, chemiosmotic membrane potential may be saved for ATP production. Thauer's group subsequently showed that electron bifurcation occurs (4). However, whether electron bifurcation directly couples heterodisulfide reduction to the first step in methanogenesis has not been proven, nor has it been shown whether formate can replace hydrogen as the electron donor to Hdr. In addition, since growth yields vary markedly, the efficiency of energy conservation is also variable. Uncoupling of methanogenesis from growth could be useful in hydrogen production technologies.

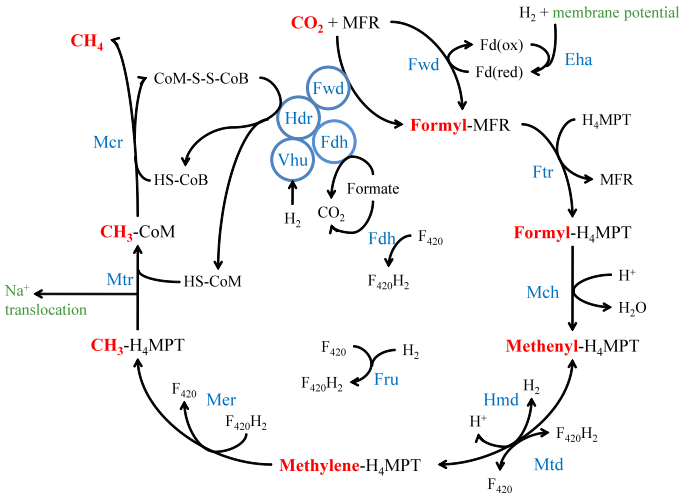

FIGURE 1. Methanogenic pathway showing the electron bifurcating Hdr complex and direct association of formate dehydrogenase.

Progress Report

Protein complexing suggests electron bifurcation and electron delivery from formate to heterodisulfide reductase. By purifying Hdr from *M. maripaludis*, we discovered a protein complex that contains not only Hdr but a hydrogenase (Vhu), a formate dehydrogenase (Fdh), and formylmethanofuran dehydrogenase (Fwd) (5). The existence of the complex supports a model of electron flow in which either hydrogen or formate directly reduce the flavin of Hdr, and electron bifurcation leads to the reduction of heterodisulfide and of CO_2 to formylmethanofuran (formyl-MFR). In addition, in a mutant deleted for Vhu, growth on hydrogen was poor but growth on formate was as good as wild type.

Genetic analysis reveals three pathways of hydrogen utilization for methanogenesis and an independent pathway of formate utilization. We have generated numerous deletion mutants in genes for hydrogenases. Only in a mutant deficient in three hydrogenases could we eliminate the ability of hydrogen to support methanogenesis. Thus, we eliminated F_{420}-reducing hydrogenase (Fru), Hdr-associated hydrogenase (Vhu), and the hydrogenase Hmd that can act in concert with Mtd to reduce F_{420} (6). This mutant required formate for growth even when hydrogen was present. It also required hydrogen, and evidence to be presented suggests that the hydrogen requirement is restricted to biosynthesis. This result alters the previous view in which methanogenesis from formate required production of hydrogen from formate. Instead, it now appears that formate can support methanogenesis directly (5, 7). In addition, the multiple-hydrogenase mutant should be unable to re-utilize for methanogenesis any hydrogen that is produced

Uncoupling of methanogenesis from growth does not depend on the energy-conserving hydrogenase Ehb. Growth yields (cell mass produced per methane produced) of *M. maripaludis* were three-fold higher under hydrogen limitation (in chemostats) compared to hydrogen excess. Thus, under hydrogen excess conditions metabolism partially uncouples from growth, enhancing prospects for growth-independent hydrogen production. Uncoupling could occur by several mechanisms, including depletion of chemiosmotic membrane potential by the energy-conserving hydrogenase Ehb (8). However, uncoupling still occurred in a *ehb* mutant.

Future Directions

Our strategy for hydrogen production from formate involves the derepression of nitrogenase in a mutant that is deficient in hydrogen re-uptake. Based on the above results, we are now ready to construct a strain that fulfills these criteria. In addition, using the purified Hdr complex we will test in vitro whether formate is indeed a direct electron donor and whether the complete pathway of electron bifurcation occurs. Finally, to maximize hydrogen production we seek to optimize the uncoupling of methanogenesis from growth, in order to direct maximal ATP to nitrogenase. To this end, we will test several hypotheses for the basis of uncoupling.

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Publication list (including patents) acknowledging the DOE grant or contract

1. Costa KC, Wong PM, Wang T, Lie TJ, Dodsworth JA, Swanson I, et al. Protein complexing in a methanogen suggests electron bifurcation and electron delivery from formate to heterodisulfide reductase. Proc Natl Acad Sci U S A. 2010;107(24):11050-5.

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