Development of a Molecular System for Efficient Production and Maturation of Fe-only Hydrogenases

Investigators
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Introduction

We discovered that the bacterium *Shewanella oneidensis* MR-1 contains a *hydA* gene and produces hydrogen. We therefore decided to take advantage of this knowledge and use *S. oneidensis* MR-1 as a model organism for the understanding of Fe-only hydrogenase assembly, folding and maturation.

Analysis of the *S. oneidensis* genome revealed two putative hydrogenase gene clusters, *hydA* and *hyaB*. HydA is predicted to be a Fe-only membrane-associated hydrogenase while HyaB is predicted to be a Ni-Fe hydrogenase. The *hydA* gene cluster contains accessories proteins which are believed to be involved in the correct assembly and folding of the Fe-only hydrogenase. The enzymes were believed to be involved in hydrogen uptake and evolution during anaerobic growth, however, their role was never deeply investigated.. Although a structure mimics the H-cluster was recently synthesized not much is known yet about the role and function of the accessory proteins involved in assembly and insertion of metal clusters, assembly of iron-sulfur clusters and the folding of Fe-only hydrogenases. Understanding the maturation process is crucial for the design and optimization of biological hydrogen production in cyanobacterial heterocysts.

Results

Shewanella oneidensis evolves hydrogen

When *S. oneidensis* cells were grown anaerobically in mineral medium supplemented with lactate as electron donor and fumarate as electron acceptor, we discovered the formation of molecular hydrogen. The onset of hydrogen formation correlated with the depletion of fumarate and with cells entering stationary phase. A series of growth experiments was performed in order to explore which conditions affects hydrogen evolution. Significant hydrogen formation was observed from pyruvate. Hydrogen evolution was correlated with the appearance of formate. Based on those results and on genome sequence, we hypothesize that hydrogen is formed from formate by a hydrogen formate lyase, where formate is derived from pyruvate. Hydrogen formate lyase is a multi-component membrane associated complex, comprising formate dehydrogenase and hydrogenase HydA.

Mutants lacking genes encoding for hydrogenases

We constructed in-frame deletion mutants of *hydA*, *hyaB* and a *hydA/hyaB* double mutant. The mutants were tested both in growth experiments using lactate: fumarate and pyruvate: fumarate and in cell suspension experiments with lactate, pyruvate and formate

in the absence of an electron acceptor. In the *in*-vivo growth experiments in the presence of excess pyruvate, hydrogen production in *ΔhydA* and *ΔhyaB* mutants was reduced only to 25% of the yield compared with the wild type. Evolution of hydrogen in the *ΔhyaB* mutant started 28 hrs after growth began, while the *ΔhydA* mutant started after about 24 hours. Hydrogen evolution was not detected from the double mutant *ΔhydA/ΔhyaB*, suggesting that HydA and HyaB are the only enzymes required for hydrogen production in *S. oneidensis*.