Ph.D thesis

Connection between sulfur metabolism and hydrogenases in a purple sulfur bacterium

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Introduction

In the near future fossil energy carriers become less available leading to difficulties in the energy supply. Moreover, the environmental impact of the fuels must also be taken into consideration. Biohydrogen might be one of the most promising (bio)fuel candidate since its usage/combustion/oxidation results in water. Biohydrogen could be produced via dark and photo-fermentation using hydrogenases and/or nitrogenases. The photoautotrophic hydrogen production would be the ideal solution applying anoxic organisms for sustainable hydrogen production. Purple sulfur bacteria uses sulfur compounds as photosynthetic electron donors and cyclic electron transport for energy generation. They cannot cleave water, thus can't produce oxygen.

Our model organism, *Thiocapsa roseopersicina* BBS is a photosynthetic purple sulfur bacterium, which is able to produce hydrogen from inorganic and organic compounds via its nitrogenase and hydrogenase enzymes. In this strain, there are four active [NiFe] hydrogenases. Two of them are membrane-associated: Hyn and the hydrogen uptake enzyme: Hup. The Hyn hydrogenase is a bidirectional enzyme and it has remarkable stability under extreme conditions. It consists of two core subunits and two electron transfer subunits, named as Isp1 and Isp2. Isp1 is predicted to be a transmembrane protein, while Isp2 seems to be a membrane- associated enzyme with cytoplasmic orientation. The other two hydrogenases Hox1 and Hox2- are cytoplasmic NAD^+-reducing enzymes. Hox1 could produce hydrogen both under illumination and in the darkness, as well.

Aims of the project:

- I wish to disclose the metabolic routes, bioenergetic processes linked to the Hyn catalyzed hydrogen evolution/oxidation.
- I want to identify the electron donors and electron acceptors of Hyn hydrogenase in *T. roseopersicina*.
- I perform *in silico* examination of the metabolic pathways, which are connected to Hyn hydrogenase. Especially, I will focus on the sulfur metabolism, since these are the primary electron sources of our model organism.
- I wish to establish if the Hyn hydrogenase is coupled to the membrane redox system / photosynthetic electron transport chain?
- What is the physiological role of Isp2?
- I determine which sulfur compounds might serve as electron donors of Hox1 hydrogenase under photoautotrophic conditions.
- I build up an integrated electron transport model about the connection between Hyn and Hox1 hydrogenases and sulfur metabolism / membrane redox system.

Methods:

BLAST and ClustalX softwares were used for similarity search of predicted gene products. Standard DNA manipulation techniques were applied for plasmid constructions, *in frame* mutagenesis and homologous complementation experiments. The determination of HynSL amount was performed by Western analysis. Hydrogen evolution and uptake were measured in the presence of various kinds of electron donors and acceptors by using gas chromatography. The hydrogen sulfide and sulfate formations were followed by gas and liquid chromatography.

Results

- Thiosulfate and elemental sulfur are the electron donors of the hydrogen evolution of the Hyn hydrogenase.
- In the case of Hyn hydrogenase, thiosulfate might be better electron donor than sulfur oxidation.
- Sulfite oxidation is not linked to the hydrogen evolution of Hyn.
- Elementary sulfur and nitrate can facilitate the hydrogen uptake of Hyn.
- The hydrogen evolution of the Hyn hydrogenase is light dependent.
- Hydrogen dependent hydrogen sulfide production could be inhibited by the Q_b site specific competitive inhibitor terbutryn, therefore there is a bidirectional connection between the photosynthetic electron transport chain and the Hyn hydrogenase.
- The proton motive force generated by the photosynthetic electron transport chain has a role in the hydrogen production of Hyn.
- Strain lacking *isp2* gene was generated by *in frame* mutagenesis.
- In the absence of Isp2, both the hydrogen evolution and the hydrogen driven hydrogen sulfide production were also abolished. The Isp2 is part of the electron transport chain between HynSL and membrane redox system.
- The hydrogen evolution of the Hox1 hydrogenase is linked to the thiosulfate, sulfur and sulfite oxidation.
- The competitivity between hydrogen sulfide production and hydrogen evolution of Hox1 supports the connection of Hox1 to the quinone pool.
- The role of Hox1 in the cellular metabolism might be the protection against the overreduction of the membrane redox system.

The model based on my results:

Thiosulfate and elemental sulfur assimilation donates electrons to photosynthetic reaction via periplasmic cytochromes under illumination. Electrons transferred from the Q_b site of the reaction center to an unknown redox mediator, which donates electrons to the Isp2 subunit of Hyn. Then the electrons are transferred to the catalytic subunit via Isp1 and hydrogen is formed. It was demonstrated that the H_2 production of Hyn is light dependent and partially proton motive force driven. Since apparent H_2 production could be detected even at high uncoupler concentration. H_2 uptake driven hydrogen sulfide formation experiments revealed that the electrons deriving from Hyn catalyzed H_2 oxidation can be transferred to the quinone pool via the Q_b site of reaction center. Electrons from HynSL transferred to quinone pool could be utilized for nitrate and sulfur reduction also.

In contrast to Hyn hydrogenase, the Hox1 hydrogenase could get electrons from thiosulfate assimilation, sulfur oxidation, and sulfite oxidation also under illumination. The role of Hox1 in the cellular metabolism could be the protection of the membrane redox system from the overreduction.

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Publications:

Publications connected to this work:

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Hydrogenase related metabolic networks in *Thiocapsa roseopersicina.* Roland Tengölics, Rita Béres, Zsolt Doffkay, Tünde Csata, János Orosz, Sebestyén Simonkovich, Márta Ondrésik, Edit Győri, Kornél L. Kovácsand Gábor Rákhely 10th Hydrogenase Conference Szeged,

Conference posters:

Connections between sulfur metabolism and Hyn hydrogenase of *Thiocapsa roseopersicina* BBS. Roland Tengölics, Lívia Mészáros, Zsolt Doffkay, András Tóth, Ágnes Duzs, Edit Győri, Kornél Kovács, and Gábor Rákhely. EMBO Workshop on Microbial Sulfur Metabolism.