# Metabolic versatility of [NiFe]-hydrogenases in *Thiocapsa roseopersicina*

Ph.D. Thesis

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# Introduction

Microbial hydrogen production and consumption implicate hydrogenase enzymes as biocatalysts for the reversible oxidation of molecular hydrogen. Most of the organisms - capable of metabolizing hydrogen - are prokaryotes belonging to the Bacteria and Archea domains of life. However, hydrogenases have also been identified in the subcellular organelles of eukaryotes. Hydrogenases catalyse either proton reduction or hydrogen oxidation *in vivo* in numerous metabolic contexts. $H_2$ is consumed as a source of energy and reductant during chemolithoautotrophic growth and is produced as a fermentation product during anaerobic, heterotrophic growth. Many anoxygenic phototrophs can utilize $H_2$ as an electron donor for photoautotrophic growth.

The presently known hydrogenases belong to three groups of independent phylogenetic origin. These groups are defined on the basis of their metal content: [NiFe]-hydrogenases, [FeFe]-hydrogenases and [Fe]-hydrogenases; each characterised by a distinctive functional core that is conserved within each class. The core consists of the subunits minimally required for structure and function.

The biggest and best studied class of hydrogenases are [NiFe]-hydrogenases. The basic module of a catalytically active [NiFe]-hydrogenase consists of an αβ heterodimer. The approximately 60 kDa large subunit (α-subunit) hosts the binuclear [NiFe] active site composed of a Ni, Fe, a CO and two CN ligands, while the small

subunit (β-subunit) of ca. 30 kDa hosting the Fe-S clusters. The [NiFe] active site is coordinated by four cysteines of the large subunit. Two of the thiolate groups (provided by Cys68 and Cys533) form a bridge between the two metals. The small subunit contains up to three linearly arranged cubic Fe-S clusters, which conduct electrons between the $H_2$-activating center and the (physiological) electron acceptor/donor of hydrogenase. The proximal [4Fe-4S] cluster could directly exchange electrons with the catalytic site, they are both buried deep in the protein.

*Thiocapsa roseopersicina* BBS is a purple sulphur photosynthetic bacterium belonging to the Chromatiaceae family.

*T. roseopersicina* was known to harbour two membrane-associated (HynSL and HupSL) and one soluble (HoxEFUYH) [NiFe]-hydrogenases differing in their subunit composition, localisation, stability and *in vivo* function. Whole genome sequencing of *T. roseopersicina* revealed the coding sequence of an additional soluble hydrogenase enzyme. Therefore, this strain has one of the most complex hydrogen metabolisms, hydrogenase network. Our aim is to disclose the molecular background of the biosynthesis of the individual hydrogenases, their expression regulation, their metabolic linkages for understanding their physiological role and metabolic interactions. Within this global project, the aims of this work were the followings:

Examination of the role of the HupC protein in order to obtain better insight into the *in vivo* function of HupSL hydrogenase.

Characterisation of the Hox2 hydrogenase by studying domain organisation, examining *in vivo* and *in vitro* activity properties, disclosing its physiological function.

Comparison and analysis of the possible *in vivo* roles of the Hox1 and Hox2 bidirectional hydrogenases.

# Methods

DNA manipulations and analyses were performed according to standard techniques and the specifications of the manufacturers. Plasmids were transferred into *E. coli* by transformation and electroporation and into *T. roseopersicina* via conjugation. Deletion analyses, different types of activity measurements, real-time quantitative PCR experiments were performed for the functional characterization of the various hydrogenases. As a first step, deletions were created in *hupC* and *hox2H* genes, and the mutations were complemented by the appropriate genes carried by plasmids. Detailed characterization of hydrogenase uptake and evolution activities of cultured and disrupted cells was used to characterize the various mutant phenotypes. Quantitative PCR approach was used to follow the expression level of the *hox2* genes in various strains (wild-type, triple hydrogenase mutant GB112131). Systematic measurements of the thiosulphate and glucose levels also provided essential information on the physiological function of Hox2 hydrogenase.

# Results

Multiple [NiFe]-hydrogenases participate in the hydrogen metabolism of the purple sulphur photosynthetic bacterium *Thiocapsa roseopersicina*. These enzymes are connected to various bioenergetic/redox processes of the cells. Functional investigation of the electron transfer subunit (HupC) of the membrane-associated Hup hydrogenase and detailed characterisation of the recently identified Hox2 hydrogenase were in the focus of my PhD work.
The following summarizing statements are concluded based on my results:

I created *ΔhupC* in frame deletion mutant strain of *T. roseopersicina* in order to examine the possible role of this protein. HupC was shown to be part of the *in vivo* functional Hup hydrogenase complex, as an electron transfer subunit. It is also required for the stability and membrane association of the Hup enzyme.

Lack of the HupC protein resulted in elevated expression level of the *hupSL* genes. I postulated that in the absence of HupC, the electron transfer, from the Hup hydrogenase to the quinone pool of the cell is blocked leading to a more oxidised quinone pool which

upregulates the expression of the electron-donating Hup hydrogenase.

I demonstrated that the amount of the available external electron source (thiosulphate) in the growth medium has a regulatory effect on Hup expression, which is likely realised through the quinone pool.

Further hydrogenase structural and accessory genes were discovered in the genome of *T. roseopersicina* encoding for heterotetrameric cytoplasmic [NiFe]-hydrogenase (Hox2FUYH) and a supposedly Hox2 specific endopeptidase (Hox2W).

I optimized the growth conditions for the *in vivo* Hox2 hydrogenase activity. The simultaneous presence of low thiosulphate concentration and glucose is required and sufficient for the *in vivo* metabolic activation of the Hox2 hydrogenase. Hox2 mediated hydrogen production appears at the early stationary phase of photomixotrophic growth.

The role of thiosulphate and glucose was investigated by following their concentrations during the life cycle of the cells in line with Hox2 activity and expression pattern. The results indicate that thiosulphate is the major electron source utilized by the cells at the logarithmic growth stage. Glucose metabolism takes over at the

stationary phase and donates electrons to the previously accumulated Hox2 hydrogenase.

I proved that Hox2 is a real bidirectional hydrogenase - able to catalyse *in vivo* hydrogen production and consumption. It is located in the cytoplasm and able to catalyse NADH- but not NADPH-dependent hydrogen evolution *in vitro*.

Expression analysis revealed dissimilar expression of the genes encoding for the hydrogenase (*hox2YH*) and diaphorase (*hox2FU*) dimers of the Hox2 hydrogenase, which implies the presence of two transcriptional units and/or distinct stability of the mRNA harbouring the *hox2FU* and *hox2YH* genes. The considerably low level of *hox2YH* transcript may explain the low hydrogen producing capability of Hox2 hydrogenase. In addition, the transcript levels of the *hox2* genes are different in the wild-type and triple (Δ*hyn*,Δ*hup*,Δ*hox1H*) hydrogenase mutant strains indicating an interplay between the hydrogenases present in the cell.

I investigated the structural differences between the two Hox-type hydrogenases representing a heteropentameric and heterotetrameric forms of this group of [NiFe]-hydrogenases. I pointed out the novelty of the simultaneous presence of two functional Hox-type hydrogenases of various subunit composition in an organism.

I also observed the functional distinction of these enzymes. Both the hydrogen producing activity of the Hox1 hydrogenase and the expression level of the *hox1YH* genes are considerably higher than that of Hox2/*hox2YH*. Nevertheless, the expression level of the diaphorase encoding genes did not differ from eahother.

I have shown, that Hox2 gets metabolically active after the start of glycolysis, while Hox1 is able to use both glucose and thiosulphate as electron source for hydrogen production.

# Publications

## Publications covering the thesis

**Maróti, J., Farkas, A., Nagy, I. K., Maróti, G., Kondorosi, É., Rákhely, G. and Kovács, K. L. (2010)** Second soluble Hox-type NiFe enzyme completes the hydrogenase set in *Thiocapsa roseopersicina* BBS. *Appl. Environ. Microbiol*. (In press)

**Palágyi-Mészáros, L. S., Maróti, J., Latinovics, D., Balogh, T., Klement, E., Medzihradszky, K.F., Rákhely, G. and Kovács, K. L. (2009)** Electron-transfer subunits of the NiFe hydrogenases in *Thiocapsa roseopersicina* BBS. *FEBS J.* **276(1)**:164-174.

## Further publications

**Maróti, G., Rákhely, G., Maróti, J., Dorogházi, E., Klement, E., Medzihradszky, F. K. and Kovács K. L.** (**2010)** Specificity and selectivity of HypC chaperonins and endopeptidases in the molecular assembly machinery of [NiFe] hydrogenases of *Thiocapsa roseopersicina*. *Int. J. Hydrogen Energy* **35**: 3358-3370.

**Kovács, K. L., Kovács, A. T., Maróti, G., Mészáros, L. S., Balogh, J., Latinovics, D., Fülöp, A., Dávid, R., Dorogházi, E. and Rákhely, G. (2005)** The hydrogenases of *Thiocapsa roseopersicina*. *Biochem. Soc. Trans*. **33**:61-63.

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**Kovács, K. L., Fodor, B., Kovács, Á. T., Csanádi, Gy., Maróti, G., Balogh, J., Arvani, S. and Rákhely, G. (2002)** Hydrogenases, accessory genes and the regulation of [NiFe] hydrogenase biosynthesis in *Thiocapsa roseopersicina*. *Int. J. Hydrogen Energy*, **27**:1463-1469.

**Kovács, K. L., Kovács, Á. T., Maróti, G., Bagi, Z., Csanádi, Gy., Perei, K., Bálint, B., Balogh, J., Fülöp, A., Mészáros, L. S., Tóth, A., Dávid, R., Latinovics, D., Varga, A. and Rákhely, G. (2004**) Improvement of biohydrogen production and intensification of biogas formation. *Rev. Environ. Sci. Biotechnol*. **3(4)**: 321-330.