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The connection between structure and function of electron-transfer subunits in Thiocapsa roseopersicina BBS

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Hydrogen can be considered as a potential renewable alternative fuel replacing fossil resources. Hydrogen gas can be produced by biological systems via hydrogenase or nitrogenase enzymes. Numerous phototrophic microbes are able to capture light energy and produce hydrogen. Hydrogenases are catalyzing the following simple reaction: $2H^+ + 2e^- \leftrightarrow H_2$ The cells dispose excess electrons through hydrogen production catalyzed by hydrogenases, while oxidation of molecular hydrogen mostly provides electrons for reductive and/or various energy conserving processes, such as respiration. [NiFe] hydrogenases consist of a large and a small subunit. The large subunit contains the binuclear metallocenter which is the active site of the enzyme. The small subunit is responsible for the electron transport between the active center and the surface of the enzyme.

Thiocapsa roseopersicina BBS is an anaerobic purple sulfur phototrophic bacterium isolated from the North Sea. It can grow on inorganic carbonate with reduced sulphur compounds (sulphide, thiosulphide or elementary sulphur) as electron donors, but it can also utilize organic compounds (e.g. sugar and acetate).

There are two membrane-bound (HynSL and HupSL) and two soluble (HoxEFUYH, HoxFUYH) [NiFe] hydrogenases in *T. roseopersicina*. HynSL shows extraordinary heat stability and it is resistant to oxygen inactivation. The arrangement of the structural genes coding for this enzyme differs from the organization of common hydrogenases because the genes of small and large subunits are interrupted by two ORFs: *isp1* and *isp2* (Rákhely et al. 1998).

In silico sequence analysis disclosed that Isp1 contains five transmembrane helices and a heme b binding motif, while Isp2 resembles the heterodisulfide reductases and contains Fe-S clusters. Therefore, they probably play an electron transfer role from/to the Hyn enzyme. Both proteins have been shown to be important for the function of the HynSL enzyme *in vivo* but neither of them is required for its expression or *in vitro* activity (Palágyi-Mészáros et al. 2009). In the *isp1,2* mutant strain, the *in vivo* H_2-producing activity of the the Hyn hydrogenase was completely lost while its *in vivo* H_2 uptake activity was dramatically decreased. The exact physiological role of the Isp proteins in the organism is still unknown.

The aim of my project is to disclose the physiologcial role of the Isp proteins and to get deeper insight into the molecular details of their function. In order to study the essential residues in these subunit, Isp1 and Isp2 proteins were (over)expressed in homologous host and purified via their His/Strep/Flag tags or by immunoprecipitation with Isp2 polyclonal antibody. The *in silico* analysis of Isp1 protein revealed 18 conserved amino acids in the primary sequence: four of them might have role in binding of the b-type heme, the function of the other 14 amino acids is still unknown. My aim was to examine the role of these amino acids in the function of Isp1 protein. Therefore, using a vector which contains the *hynS-isp1-isp2-hynL* operon of *T. roseopersicina*, each conserved amino acid was replaced by another one of distinct properties. The mutant genes were transferred back into the strain and the effect of the mutations in Isp1 was monitored via the activity of Hyn hydrogenase. Beside the histidines (His83, 96, 180 and 198) which are involved in the heme binding, other residues were also identified which are essential or important for the physiological function of the Isp1 protein.

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Rakhely G, Colbeau A, Garin J, Vignais PM, Kovacs KL (1998) Unusual organization of the genes coding for HydSL, the stable [NiFe]hydrogenase in the photosynthetic bacterium Thiocapsa roseopersicina BBS. J Bacteriol 180:1460-1465.

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The morphogenic role of brassinosteroid perception in *Arabidopsis*

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Brassinosteroids (BRs) are steroidal phytohormones that control multiple essential functions during plant development. In *Arabidopsis thaliana*, bioactive BRs are perceived at the cell surface by the extracellular domain of the plasmamembrane-localized leucine-rich repeat receptor kinase (LRR-RK) BRI1. Upon binding the hormone, this receptor initiates a phosphorylation cascade, which results in the stabilization of the BRZ transcription factors that activate or repress BR-responsive genes. Binding of the hormone facilitates heterodimerization of BRI1 with its BAK1 co-receptor, another LRR-RK, and initiates transphosphorylational self activation of the receptor complex. BRI1 was proposed to be constitutively expressed and uniformly distributed within the plant, assuming that BR responses depend mainly on local