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Analysis of a structural motif in the membrane-associated [NiFe] hydrogenases

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Hydrogenases, catalyzing the following reaction: $2H^+ + 2e \Leftrightarrow H_2$, are harbored by numerous microorganisms. The cells dispose excess electrons through hydrogen production accomplished by hydrogenases, while consumption of the molecular hydrogen mostly provides electron source for various energy conserving processes, such as respiration. Sometimes, hydrogen can be the sole energy source for the cell growth. Hydrogenases are distinguished according to the metal content in their active center: they are classified as [NiFe], [FeFe] and [Fe] enzymes (Vignais et al. 2004). Minimally, a [NiFe] hydrogenase is composed of a large and a small subunit and they can be associated to the membrane or localized in the cytoplasm. The large subunit contains a binuclear metallocenter, while the small subunit hosting the Fe-S clusters, which conduct the electrons between the H_2-activating center and the surface of the protein.

Our model organism, the purple sulfur photosynthetic bacterium *Thiocapsa roseopersicina* BBS contains at least four active [NiFe] hydrogenases. The HynSL and the HupSL enzymes are attached to the cell membrane, while Hox1YH and Hox2YH are apparently localized in the cytoplasm (Kovacs et al. 2005).

There are several conserved motifs in the sequence of the hydrogenases, which are characteristic for these enzymes. These motifs have very important role for example in coordination of the metals of the active centre, in electron transfer, in interaction with other proteins or in translocation of the fully folded protein (Vignais et al. 2007).

We have noticed a highly conserved histidine-rich region with unknown function in the large subunit of [NiFe] hydrogenases. The HxHxxHxxHxH sequence occurs in the large subunit of all membrane-bound hydrogenases, but only two of these conserved histidines are present in the soluble hydrogenases.

In order to identify the function of this motif, mutant strains were made by site-directed point mutagenesis and their biochemical properties were characterized. The *in vivo* and *in vitro* activity measurements showed that the activity was influenced dramatically only in one of the mutants due to the replacement of the His residue with Ala. Nevertheless, this enzyme still remained in the membrane.

Western hybridization experiments were applied to investigate the proteolytic stability of the enzymes. It was found that the strongly reduced activity of the mutant hydrogenase could not be derived from the destabilization of the protein.

The oxygen sensitivity of the single amino acid mutant and the wild type protein was also compared for explaining the background of significant *in vitro* and *in vivo* activity loss in the mutant strain. The crude extracts from the wild type and mutant cells obtained in anaerobe box and on air was used for *in vitro* spectroscopic measurements, but the ratio of the activities of the wild type and the mutant protein was nearly the same independently on the environment of the cell destruction.

For further biochemical and biophysical investigations, large amount of enzymes have to be purified. First, a HynS-Strep-II Tagged fusion protein was constructed to purify the non-mutant HynSL enzyme by affinity chromatography. However the amount of the purified protein was not enough for further spectroscopic experiments and the specific activity of the Strep-II fused hydrogenase was lower as compared to the natural enzyme. We continued with the standard biochemical techniques and used fast protein liquid chromatography (FPLC) for purification of the wild type and mutant protein in large scale. These experiments are in progress and the first results are very auspicious.

For getting a complete picture about the structure-function relationship in HynSL hydrogenase, other conserved residues are being investigated, as well. From biotechnological point of view thermostability and oxygen tolerance of the hydrogenases are two crucial properties. In order to improve these properties of the enzymes, identification and functional characterization of candidate sequences potentially conferring these beneficial properties to the enzymes are to be done.

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Mechanistic insights into the role of translesion synthesis and its effect on genome stability

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REV1 is a Y-family DNA polymerase. REV1 proteins contain a BRCT domain, which is important in protein-protein interactions. A suggested role for REV1 protein is as a scaffold that recruits DNA polymerases involved in translesion synthesis (TLS) of damaged DNA.