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DISSERTATION SUMMARY

Purification and examination of Hox hydrogenase in *Thiocapsa roseopersicina*

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Hydrogenases catalyze the enzymatic redox reaction $H_2 \leftrightarrow 2H^+ + 2e^-$. The purple sulfur phototrophic bacteria *Thiocapsa roseopersicina* contains several hydrogenases. HynSL is the most stabile, membrane bound, bidirectional hydrogenase. HupSL is a membrane bound H_2 uptake hydrogenase responsible for H_2 consumption produced by nitrogenase enzyme complex. The heteropentameric bidirectional HoxEFUHY hydrogenase, situated in the cytoplasm and consists of a hydrogenase part (HoxHY) and a diaphorase part (HoxEFU) which catalyzes the reduction of NAD^+.

The HoxEFUHY in *Thiocapsa roseopersicina* is a NiFe hydrogenase. These enzymes consist of a large subunit containing the catalytic center with Ni-Fe atoms, coordinated by cysteines and unique CO and CN ligands and a small subunit, responsible for e^- transport, containing Fe-S clusters. The maturation of the enzymes is influenced by several proteins, namely: HypA and HypB are needed for Ni and Fe incorporation, HypC is a chaperon, HypD and HypE is necessary for the transportation of Ni, Fe atoms, and HypF involved in the formation of CO and CN ligands. HoxW is a protease responsible for a C-terminal cleavage of the large subunit.

The genes encoding the HoxEFUHY are well known (Rákhely et al. 2004), but the enzyme itself needs to be investigated. The aim of my project was the purification and the characterization of the enzyme complex. In the first attempt the classical chromatographycal methods were used, and optimized. The chosen methods were cheramic hydroxiapatite (CHT), ion exchange (IEX), hydrophobic (HIC), and gel filtration (GF) chromatography. Purification from the soluble protein fraction, a four-step chromatography protocol (CHT→IEX→GF→HIC) was developed. The final fraction showed H_2 evolving activity, but Hox subunits could not be detected.

The enzyme was stable and easily stored after CHT but after IEX and HIC stability decreased dramatically, and activity dropped considerably. Addition of different stabilizing agents (glycerol, NaCl, detergents, reducing agents) or hypothetical cofactors (FMN, NAD, NADH) to the purified enzyme was not effective. Storage tests with the purified enzyme under special conditions - anaerobically, or in H_2 atmosphere - had no positive results.

The next approach was the affinity purification of the Hox hydrogenase using flag strepII tag fused to one of the subunits. Fusing the tag to the HoxH N-terminal end cripples the activity of the enzyme, while the C-terminal end of the large subunit processed by the HoxW. HoxE tagged construct was another alternative attempt, because HoxE is not important for in vitro activity and in vivo activity of the HoxE mutant could be reconstituted by a plasmid containing Flag-StrepII tagged HoxE. Affinity purification of the C-terminal tagged HoxE Thiocapsa strain resulted in the identification of the five Hox subunits by MALDI TOF MS. Again, most of activity disappeared during the purification procedure. Gel electrophoresis experiments showed that bands of different subunits were detected only in the fresh samples. Now there is another - HoxY C-terminal tagged - construct under construction.

It is still unclear what is needed to keep the active state of the enzyme during purification. In the strongest homologue Hox - among Cyanobacterias - similar problems have been reported. Active enzymes are purified only from Ralstonia eutropha and Rhodococcus opacus, but they are distant relatives and their Hox enzymes have different subunit structure. The stability of Hox must be improved, because pure and active Hox protein is needed for studies on substrates, cofactors, catalytic center and function of subunits.

References

Rákhely G, Kovács AT, Maróti G, Fodor BD, Csanádi G, Latinovics D, Kovács KL (2004) Cyanobacterial-type, heteropentameric, NAD^+-reducing NiFe hydrogenase in the purple sulfur photosynthetic bacterium Thiocapsa roseopersicina. Appl Environ Microbiol 70(2):722-728.

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