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DISSERTATION SUMMARY

Studies on hydrogen metabolism of hyperthermophilic *Thermococcus litoralis*

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Thermococcus litoralis is a hyperthermophilic archaeon growing optimally at 85ºC. In contrast with the majority of *Thermococcales* species, which are obligately dependent on peptides and sulfur, *T. litoralis* is able to utilize both peptides and carbohydrates as sole carbon and energy source. Sulfur is not necessary for its growth, but has positive effect on the cell yield, likely due to a bioenergetics aspect. The primary end-products of the fermentative metabolism of *T. litoralis* are acetate, CO_2 and H_2 (or H_2S). The cells remove the excess electrons formed during fermentative metabolism via H_2 with the aim of hydrogenases. However, in the presence of Sº, H_2S is formed instead of H_2. The hydrogenases seem to participate in the sulfur reduction as well.

So far, a cytoplasmic, heterotetrameric [NiFe] hydrogenase (Hyh1) has been characterized in *T. litoralis*. Recently, in a related strain, *P. furiosus*, two other [NiFe] hydrogenases were identified, and their genes were fished out from its genome. In *T. litoralis*, we have also found the genes coding for proteins corresponding to the γ-subunit of soluble hydrogenase II (Hyh2) and the α-subunit of H_2-evolving, membrane-bound hydrogenase complex (Mbh) described in *P. furiosus*.

Our aim was to clarify the physiological role of the [NiFe] hydrogenases in *T. litoralis*. The analysis of *T. litoralis* hydrogenase mutant strains would have allowed us to determine the function of the hydrogenases. However, there were no usable genetic tools for these microorganisms.

So, we intended to develop a genetic system based on antibiotic selection for *Thermococcales* cells. We have found, that puromycin was effective against *T. litoralis* cells. The lethal concentration of this antibiotic was about 15 µg/ml. In other microbes the resistance of cells against puromycin was provided by puromycin N-acetyltransferase (Pac), which was a moderately thermostable enzyme. A self-replicating vector construction was prepared, which contained the *pac* gene between the promoter and terminator region of the glutamate dehydrogenase gene of *P. furiosus* and a replication origin of a plasmid isolated from a *Pyrococcus* species. Many different transformation strategies were attempted to introduce the vector to the cells, for example, chemically-induced transformation, electroporation, transformation with liposomes, but so far these attempts were unsuccessful. However, there are other alternative protocols, which will be used in the near future.

Another approach to determine the function of the hydrogenases is the investigation of their biosynthesis in the function of the different type of fermentative metabolism of the cells. The regulation of the expression of the hydrogenases has been studied in cells growing on various carbon sources in the presence or absence of Sº.

T. litoralis cells have been cultured in well-defined media, which were occasionally supplemented with maltose and/or enzymatically-hydrolyzed casein and/or Sº. The effect of these various conditions on the hydrogenases were examined at the level of transcription, translation and the active enzymes.

The specific H_2-evolution activity of whole cells depended on the growth phase of culture. In the presence of Sº, the H_2-evolution activity was increased, if maltose or peptides were included in the medium. However, the Sº decreased the specific H_2-evolution activity of *T. litoralis* cells grown in defined medium without maltose or peptides. Western blot analysis revealed significantly less amount of Hyh1 in cells grown in the presence of sulfur. Furthermore, the relative amount of Hyh1 proteins was notably higher in *T. litoralis* cells grown without peptides or carbohydrates, than with these supplements. Similar conclusion could be obtained from preliminary real-time RT-PCR experiments, where expression of the Hyh1 was measured at transcription level. Further RT-PCR studies are being performed to confirm these data, and characterize the environmental factors and the multi-sided transcriptional regulatory mechanism controlling the activities of the various hydrogenases. This might lead to understand the physiological roles of each enzyme in more details.

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