HYDROGENASE ENZYMES IN *METHYLOCOCCUS CAPSULATUS* (BATH)

A thesis submitted by

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INTRODUCTION

Methane is the most stable volatile carbon compound in the anaerobic environment and it is a very important intermediate in the reaction that eventually leads to the mineralization of organic matter.
Methane escapes from anaerobic environments to the atmosphere when it is not oxidised by methanotrophs.
The release of methane to the atmosphere contributes in an increased rate of global warming and causes other changes in the chemical composition of the atmosphere.
Today, 60% of the methane emission is related to human activity, and the value increases continuously.

Methanotrophs are unique in their ability to utilise methane as a sole source of carbon and energy. Methane is oxidised to carbon dioxide via methanol, formaldehyde and formate intermediers. Methanotrophs are widespread in nature and play an important role in carbon cycling. The first step of this oxidation process is catalised by a methane monooxygenase enzyme (MMO).

The practical importance of these bacteria is remarkable because of their potential (environmental) biotechnological applications. Accumulation of toxic pollutants in nature cause serious environmental problems world-wide. One of the most hazardous group of pollutants is the halogenated hydrocarbons. The most widespread chlorinated hydrocarbon is trichloroethylene (TCE). TCE and other chlorinated hydrocarbons have a negative effect on human health because of their toxicity and carcinogenic effect.
Aerobic and anaerobic bacteria are able to degrade these compounds, but the product of the incomplete reaction is often a more carcinogenic vinyl-chloride. Obligate methanotrophs, although they need methane for their growth, are able to oxidise other organic compounds. Among the tested methanotrophs, those containing soluble methane monooxygenase in their cytoplasm can degrade halogenated hydrocarbons.
MMO enzymes require reducing power for catalysis. The *in vivo* electron donor for the sMMO is NADH. Under physiological conditions, the reducing power is supplied by the further oxidation of methanol produced by the MMO. Since biodegradation processes using MMO are cooxidation processes, alternative ways of supplying reducing power are needed.

A possible alternative is hydrogen. Hydrogenases show considerable potential for biodegradation and biotransformation, utilising their unique catalytic activities.

AIMS

Due to their special catalytic activity, the methane monooxygenase enzymes could potentially be used as catalysts in several environmentally-friendly and economical biotechnological processes. *In vitro*, the enzyme needs a cheap and clean reducing power. The hydrogen produced by hydrogenase enzymes is an effective candidate for this purpose.

Methylococcus capsulatus (Bath) is one of the most thoroughly studied methanotroph, hence it has been selected for our study.

My work aimed to study the hydrogenase enzymes, to characterise these enzymes at the protein level, as summarized below:

- Detect the hydrogenase enzymes in the bacterium
- Optimise hydrogenase activity measurements for *Methylococcus capsulatus* (Bath)
- Study the localization of hydrogenase enzymes
- Identify the structural genes of the soluble hydrogenase
- Search for membrane bound hydrogenase genes among methanotrophs
- Biochemical characterisation of the soluble hydrogenase enzyme
- Determine the function of the detected hydrogenase enzymes

METHODS

Methylococcus capsulatus was grown in fermenters or in flasks in a shaker. Hydrogenase activity was measured on whole cells, or on the soluble and on the membrane fractions separately. The activity was determined using two classical hydrogenase assays: In the hydrogen uptake activity measurement the production of the reduced dye was followed spectrophotometerically. Gas chromatography (GC) was used to determine the hydrogen produced during the hydrogen evolution activity assay. GC was used for the MMO activity measurements, too. Standard DNA manipulation techniques were empoyed in the molecular biological approaches (genomic and plasmid DNA purification, PCR reaction to amplify the appropriate DNA fragments, cloning, transformation, Southern hibridisation, etc.).

SUMMARY OF RESULTS

- Membrane bound hydrogenase enzyme activity was detected under nitrogen fixing and nitrogen-depleted conditions.

- The physiological role of the membrane bound enzyme was determined using a membrane bound hydrogenase mutant strain.

- Existence of an NAD^+-dependent hydrogenase in the bacterium was detected with activity measurements.

- The structural genes of the NAD^+-reducing hydrogenase were identified in the genome. The *M. capsulatus* putative gene products, HoxF, -U, -Y and –H, showed the highest similarity to the corresponding subunits of the soluble hydrogenase from *Ralstonia eutropha*.

- A *hoxH* mutant strain was constructed using marker exchange mutagenesis. The lack of the soluble hydrogenase activity caused no apparent phenotypical change in the bacterium.

- The basic biophysical and biochemical characteristics of the soluble enzyme were determined for future purification processes.

- It was confirmed that the activity of the membrane bound hydrogenase was essential in *in vivo* hydrogen driven MMO activity. Both sMMO and pMMO activities were uneffected in the *hoxH* mutant strain.

- Membrane bound hydrogenase genes are widespread among methanotrophic bacteria.

PUBLICATION LIST

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