Biosynthesis and metabolic context of enzymes related to photobiohydrogen production

Ph.D. Thesis

Andrea Nyilasi

Supervisors:

Dr. Gábor Rákhely
Prof. Kornél L. Kovács

Ph.D. School in Biology

Department of Biotechnology, University of Szeged
Institute of Biophysics, Biological Research Centre,
Hungarian Academy of Sciences

Szeged
2014

INTRODUCTION

These days one of the most common problems of mankind, that the production rate of fossil fuels is much slower than the rate of their consumption. Moreover liberation of energy is accompanied by large-scale pollution. Fact, that effects of anthropogen activity are responsible for climate changes. The Sun is one of the possible renewable energy sources while hydrogen could be the energy carrier of the future since it fulfills such elemental requirements, which are necessary for changing fossil fuels.

Molecular hydrogen can be produced converting solar energy by different biological systems with the help of hydrogenase and nitrogenase enzymes.

Our model organism, the photosynthetic *Thiocapsa roseopersicina* harbours four functional [NiFe] hydrogenases (Hyn, Hup, Hox1, Hox2) and a nitrogenase. [NiFe] hydrogenases are complex metalloenzymes, their sophisticated structure unable to form spontaneously but proper concerted action of accessory proteins is required. Numerous accessory proteins have been identified, as a result of research on hydrogenase maturation in *T. roseopersicina*, one of them is HupK. Its function is not clear however it is proven that its homologue HoxV in *Ralstonia eutropha* known as a scaffold: augmented with a Fe(CN)2CO moiety acts as an intermediate during the maturation pathway of themembrane-bound hydrogenase (MBH).

Comparing the sequence of the HupK protein to the sequences of HoxV and large subunit of Hup hydrogenase of *T. roseopersicina*. Two such motives can be found in which four cysteines

are highly conserved in large subunit of all membrane bound hydrogenases and form a coordination sphere surrounding the [NiFe] centre. However HupK and HoxV harbour two of these four conserved cysteine residues (Cys54 and Cys378 in HupK), which presumably bind the iron. The other two cysteines are substituted by phenylalanines (Phe51 és Phe381) in HupK. One of my aims was to elucidate the function of that highly conserved amino acids.

A diverse group of photosynthetic bacteria can utilize organic acids as carbon and energy sources. The aim of my work was to elicit whether the T. roseopersicina is suitable for that role and compare the hydrogen production of Hox1 and nitrogenase from different potential substrates (acetate, pyruvate, succinate and lactate).

METHODS

DNA manipulations and analyses were performed according to standard techniques reported in the literature and/or to the protocols given by the manufacturers. Plasmids were transferred into *E. coli* by transformation, into *T. roseopersicina* and *R. eutropha* via conjugation.

HupK and HoxV proteins were expressed in heterologous hosts (*R. eutropha* és *T. roseopersicina*) and *in vitro* hydrogenase activities were measured (in the presence of artificial electron acceptor).

Function of conserved amino acids in HupK accessory protein was studied by site-directed mutagenesis. The effects of amino acid changes on maturation of Hup were monitored via the Hup hydrogenase *in vitro* hydrogen uptake activity. The proteolytic stability of the Hup mutants was checked by Western hybridization.

The amount of hydrogen produced from organic acids (acetate, pyruvate, succinate and lactate) was quantified by gas chromatography. The thiosulphate and organic substrates contents of the media were measured by spectofotometric method and HPLC, respectively. The *in vitro* nitrogenase activity was followed by acetylene reduction assay.

RESULTS

In this study heterologous functionality of HupK (*T. roseopersicina*) accessory protein and the function of its conserved amino acids were investigated. Furthermore, the effect of different organic acids on hydrogen production was examined. The following statements have been established based on my results:

I. *hoxV* mutant strain of *R. eutropha* was complemented with the wild type *hupK* derived from *T. roseopersicina* and *in vitro* activity of the membrane-bound hydrogenase was measured. It was shown that HupK protein is functionally so conserved that is able to cooperate with the accessory proteins of *R. eutropha* during the maturation of hydrogenase complex.

II. HoxV protein was expressed successfully in the hupK mutant *T. roseopersicina* host. From the in vitro activities of Hup and Hyn hydrogenases, it can be concluded that the HoxV could help the maturation of both hydrogenases.

III. Conserved motives of HupK were replaced to amino acids with completely different properties by site-directed point mutagenesis. Subsequently, effects of amino acid changes on maturation of Hup were monitored via the Hup hydrogenase in vitro hydrogen uptake activity.

IV. I proved that Cys54 of HupK has a fundamental function since its replacement by alanine (*Tr* C54A) led to a dramatic reduction of Hup hydrogenase activity (to 4%). I confirmed the expression of C54A HupK with Western-blotting and hybridization. It is shown that the point mutation slightly influenced the proteolytic stability of the mutant protein. Consequently, this clarified that the activity change of the mutant strain could not be derived from the increased proteolysis or insufficient expression of C54A HupK.

V. I proved that the function of Cys54 cannot be substituted by either Cys51 or Cys381 through the investigation of further point mutant strains (*Tr* F51C/C54A és *Tr* C54A/F381C).

VI. Slight hydrogenase activity reduction of *Tr* C378A indicates that Cys378 of HupK is not an indispensable residue for the proper function of HupK.

VII. Activity comparison of *Tr* C378A with *Tr* C378A/F381C, and *Tr* F51C with *Tr* F51C/F381C led to the conclusion that Phe381 of HupK is essential. Furthermore, four cysteins in *Tr* F51C/F381C might bind iron so firmly that it might interfere with the transfer of the Fe(CN)2CO moiety from HupK to HupL.

VIII. I demonstrated that *T. roseopersicina* is unable to produce hydrogen by Hox1 hydrogenase enzyme from the examined organic acids.

IX. Based on the comparison of the effect of organic acids on nitrogenase I concluded that each substrate significantly increased

the amount of produced hydrogen, irrespectively of thiosulfate concentration.

X. Substrate consumption data suggests that *T. roseopersicina* can utilise acetate, pyruvate and lactate for heterotrophic growth in the presence of 2 and 8mM thiosulfate. At these thiosulfate concentrations the succinate did not influence the biomass accumulation.

XI. Among the examined organic acids, the highest hydrogen production was achieved from lactate. Nitrogenase activity measurements revealed that lactate increased both in vivo hydrogen production and in vitro ethyne reduction activity of nitrogenase.

XII. I also proved that lactate enhanced the expression of α and β subunits of nitrogenase.

PUBLICATIONS

Papers related to the thesis

(1) **Andrea Nyilasi**, Gergely Maróti, Tímea Balogh, Kornél Lajos Kovács, Gábor Rákhely (2014) Heterologous functionality and roles of conserved cysteine motifs of the [NiFe]-hydrogenase accessory protein, HupK/HoxV, International Journal of Hydrogen Energy (in press); IF: 3.548

(2) **Andrea Nyilasi**, Éva Molnos, Szabolcs Lányi, Iosif Nagy, Gábor Rákhely, Kornél L. Kovács (2013) Photofermentative production of hydrogen from organic acids by the purple sulfur bacterium Thiocapsa roseopersicina, International Journal of Hydrogen Energy 38:(14) pp. 5535-5544. IF: 3.548

Further papers

(3) Emma Szőri-Dorogházi, Gergely Maróti, Milán Szőri, **Andrea Nyilasi**, Gábor Rákhely, Kornél L. Kovács (2012) Analyses of the large subunit histidine-rich motif expose an alternative proton transfer pathway in [NiFe] hydrogenases, Plos One (4) Paper N°e34666. 11 p.; IF: 4,411

(4) **Andrea Nyilasi**, Zsolt Horváth, Kornél L. Kovács, Gábor Rákhely (2013) Hydrogen production from lactate by a purple sulfur phototrophic bacterium, Acta Microbiologica et Immunologica Hungarica 60:(Suppl. 1.) pp. 200-201.

(5) Kovács Kornél, Fülöp András, Herbel Zsófia, **Nyilasi Andrea**, Rákhely Gábor (2010) Tiszta, megújuló energia a biohidrogén, Környezetvédelem XVIII.(2):20-21

(6) **Andrea Nyilasi**, Gergely Maróti, Gábor Rákhely, Kornél L. Kovács. (2005) Investigation of HupK hydrogenase accessory protein in Thiocapsa roseopersicina, Acta Microbiologica et Immunologica Hungarica 52, 113-114.

(7) Éva Klement, Krisztina Buzás, Gergely Maróti, Barna Fodor, Ákos T. Kovács, Dóra Latinovics, Lívia Mészáros, Réka Dávid, **Andrea Nyilasi**, Judit Balogh, Gábor Rákhely, Kornél L. Kovács, Katalin F. Medzihradszky. (2005) Mass spectral identification of interacting proteins in the biosynthesis of Ni-Fe hydrogenases, Acta Microbiologica et Immunologica Hungarica 52, 75-76.

(8) **Andrea Nyilasi**, Kornél L. Kovács, Gábor Rákhely (2009) Investigation of the maturation of NiFe hydrogenases in Thiocapsa roseopersicina, Acta Biologica Szegediensis, 53 (No. 1), 70

(9) Éva Molnos, **Andrea Nyilasi**, Gábor Rákhely, Ovidiu Muntean, Kornél L. Kovács (2010) Photofermentative production of hydrogen by Thiocapsa roseopersicina from Simple organic substrates, Hungarian Journal of Industrial Chemistry, 38. (2), 117-121