Investigation of the autocatalytic function of hydrogenase enzyme

Ph.D. Thesis

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Introduction

Hydrogenases are metalloenzymes which catalyze the reversible oxidation and reduction of molecular hydrogen: $H_2 \rightleftharpoons 2\ H^+ + 2e^-$. Hydrogen uptake is coupled to energy conversation (producing NAD(P)H, methane), while hydrogen evolution is a way of releasing excess reductants. They have come into research focus 25 years ago and their research is still ongoing. Our limited sources of oil and natural gas motivate the searching of new, alternative ways to fulfill our increasing energy hunger. The hydrogen is an environment-friendly energy carrier as its oxidation results in pure water. Both hydrogen oxidation (uptake) and hydrogen reduction (evolution) activity of hydrogenases are used in biotechnological applications. There are efforts to produce of hydrogen both in *in vivo* systems using photosynthetic microorganisms and in artificial systems using immobilized hydrogenases. Hydrogenases can also be used to produce reduced cofactors, in denitrification and in treatment of waste water. They can also replace the expensive platinum in fuel cells.

To apply an enzyme with high efficiency in artificial systems, we should know about its function as much as possible, including enzyme mechanism and optimal reaction conditions. Despite the fact, that hydrogenases are known since 1930s, their structure and function are still under study. Usually they are extremely oxygen sensitive and they do not follow the classical Michaelis-Menten

kinetics as it turned out from early activity assays. Recently their kinetic properties were explained by an autocatalytic mechanism.

Aims of the study

My goal was to prove the prion type autocatalysis of Hyn hydrogenase from *Thiocapsa roseopersicina* and to determine the factors affecting the autocatalytic step by investigating the autocatalytic reaction pattern in two dimensions under different reaction conditions in a thin layer reaction system.

I have planned the following experiments:

1. setting up of a new thin layer reaction system in order to precisely determine the propagation of autocatalytic front,
2. measurement of the dependence of front propagation on enzyme concentrations to prove the protein nature of autocatalyst (prion type autocatalysis),
3. demonstration of the existence of a protein-protein complex as a result of an autocatalytic protein-protein interaction by using a protein cross-linking method (PICUP, photo-induces cross-linking of unmodified proteins),
4. demonstration of a conformational change during hydrogenase activation by dynamic light scattering (DLS) method,

5. examination of the effect of salts from Hofmeister series (neutral, kosmotropic, chaotropic) on autocatalytic front propagation and on the autocatalytic reaction pattern,
6. measuring the dependence of front propagation on electron acceptor concentration, hydrogen concentration and proton concentration,
7. determine if there are specific reaction conditions under which the reaction shows oscillation behaviour,
8. building new kinetic models to place the autocatalytic step along the reaction cycle.

Methods

I investigated the dependence of autocatalytic front propagation and autocatalytic pattern on enzyme concentration, electron acceptor concentration, hydrogen concentration and on different salts in a new thin layer reaction system. Front velocities were determined by a software developed in MATLAB by Dr. Csaba Bagyinka.

I used a known protein cross-linking method (PICUP) under optimized conditions.

The possibility of a conformational change during hydrogenase activation was investigated by a dynamic light scattering instrument and data were analyzed by the instrument's own software.

CVOD and MATLAB software were used to solve the equations for kinetic models.

Results

A new thin layer reaction system was set and I could determine precisely the velocity of autocatalytic front propagation.

In the experiments aiming to prove prion type autocatalysis I had the following results:

- in thin layer experiments front velocity increased as a square root function by increasing enzyme concentration, which proves the role of hydrogenase as autocatalyst,
- I demonstrated the existence of a protein complex during the enzyme activation, which is probably the result of two interacting small subunits of the hydrogenase,
- in DLS experiments I could not detect significant changes in the hydrogenase conformation during activation, so the conformation change should not significantly alter the hydrodynamic radius of the molecule,
- all type of salts (kosmotropic, chaotropic and neutral) influenced the autocatalytic pattern, but only the chaotropic salt, which favors protein conformation change, decreased significantly the front velocity, which result suggests a small conformational change during the autocatalytic step.

I investigated the dependence of front velocity on different conditions:

- increasing electron acceptor concentration caused a decreasing front velocity which implies that the substrate reacts directly with the autocatalyst form of the enzyme and this interaction should take place through the distal FeS cluster in the hydrogenase,
- different hydrogen (substrate) concentration did not caused significant change in front velocity,
- proton concentration (pH) resulted in an interesting and hardly explicable changes, which can be explained by dual roles of protons, first as products of the autocatalytic reaction and second as factors affecting protein structure.

Focusing on these new experimental observations a modified autocatalytic triangular enzyme model was constructed. Two generic models were presented: first one considered the autocatalytic step within the enzymatic cycle, while in the second one it was outside the enzyme cycle. According to *in silico* analysis both models supported experimental observations.

I could generate damped oscillations in the hydrogenase reaction, which is an important characteristic of an autocatalytic process.

Publications related to the thesis

Bodó G, Branca RM, Tóth Á, Horváth D & Bagyinka Cs (2009) Concentration-dependent front velocity of the autocatalytic hydrogenase reaction. *Biophys J* **96**, 4976-4983.

Ősz J, **Bodó G**, Branca RM & Bagyinka Cs (2005) Theoretical calculations on hydrogenase kinetics: explanation of the lag phase and the enzyme concentration dependence of the activity of hydrogenase uptake. *Biophys J* **89**, 1957-1964.

Other publications

Branca RM, **Bodó G**, Várkonyi Zs, Debreczeny M, Ősz J & Bagyinka Cs (2007) Oxygen and temperature-dependent structural and redox changes in a novel cytochrome c(4) from the purple sulfur photosynthetic bacterium *Thiocapsa roseopersicina*. *Arch Biochem Biophys* **467**, 174-184.

Branca RM, **Bodó G**, Bagyinka Cs & Prókai L (2007) De novo sequencing of a 21-kDa cytochrome c4 from *Thiocapsa roseopersicina* by nanoelectrospray ionization ion-trap and Fourier-transform ion-cyclotron resonance mass spectrometry. *J Mass Spectrom* **42**, 1569-1582.

Tomčová I, Branca RM, **Bodó G**, Bagyinka Cs & Smatanová IK (2006) Cross-crystallization method used for the crystallization and preliminary diffraction analysis of a novel di-haem cytochrome c4. *Acta Crystallogr Sect F Struct Biol Cryst Commun* **62**, 820-824.

Conference abstracts

Talks

Cytochrome c_4 from *Thiocapsa roseopersicina*

Rui M Branca, **Gabriella Bodó**, Zsuzsanna Várkonyi, Judit Ősz, Mónika Debreczeny, Csaba Bagyinka

8th International Conference on Membrane Redox Systems 2006, Szeged, Hungary

Protein-protein interaction during autocatalytic reaction cycle of hydrogenase enzyme from purple photosynthetic bacteria *Thiocapsa roseopersicina*

Gabriella Bodó, Judit Ősz, Csaba Bagyinka

Dynamics Days 2003, Palma de Mallorca, Spain

Experimental and theoretical evidence for the autocatalytic reaction cycle of hydrogenase enzyme from *Thiocapsa roseopersicina*

Judit Ősz, **Gabriella Bodó**, Csaba Bagyinka

Dynamics Days 2003, Palma de Mallorca, Spain

Poster

Autocatalytic reaction of hydrogenase from *Thiocapsa roseopersicina*

Gabriella Pankotai-Bodó, Rui M Branca, Ágota Tóth, Dezső Horváth, Csaba Bagyinka

33rd FEBS Congress - 11th IUBMB Conference 2008, Athens, GREECE

(2008) *Febs Journal* **275**, 204-204.

The autocatalytic reaction cycle of hydrogenase: evidences, models and possible physiological importance

Gabriella Pankotai-Bodó, Rui M Branca, Ágota Tóth, Dezső Horváth, Csaba Bagyinka

2008 Gordon Research Conference on *Oscillations & Dynamic Instabilities in Chemical Systems*, Waterville, ME, USA

The autocatalytic reaction of hydrogenase enzyme

Judit Ősz, **Gabriella Bodó**, Rui M. Branca, Csaba Bagyinka

30th FEBS Congress - 9th IUBMB Conference 2005, Budapest, Hungary

(2005) *Febs Journal* **272**, 257-258.

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