PROJECT PROPOSAL
for applicants for Ph.D. fellowships

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project title: AUTOCATALYTIC ENZYME REACTION

PROJECT SUMMARY
Hydrogenases are metalloenzymes that catalyze the reversible oxidation and reduction of molecular hydrogen, \( \text{H}_2 \rightleftharpoons 2\text{p}^+ + 2\text{e}^- \). There are two autocatalytic reaction steps in the \( \text{H}_2 \)-oxidizing reaction of hydrogenase. One autocatalytic reaction occurs when the enzyme is mainly in inactive forms. The most obvious sign of this autocatalytic step is that the reaction starts from distinct points. The reaction-diffusion front is direct evidence of an autocatalytic reaction. The second autocatalytic step is located in the enzyme cycle. Its presence is supported by the facts that the Ni-R form is exclusively diamagnetic and the steady-state concentration of the product of the \( \text{H}_2 \)-oxidizing reaction displays a strong enzyme concentration dependence. The student will study the details of these autocatalytic reaction steps; identify their localization in the reaction cycle, identify the autocatalyst etc. using different biochemical and spectroscopic methods.

BACKGROUND OF THE STUDY
Hydrogenases are metalloenzymes that catalyze the reversible oxidation and reduction of molecular hydrogen. The active center of the enzyme is a very special [Ni-Fe] center, and it also contains three [FeS] clusters. Their hydrogen oxidation (hydrogen uptake) activity is important, because hydrogenase could replace the expensive platinum electrodes in fuel cells.

Previous experimental results and theoretical considerations led to the conclusion that there are two autocatalytic reaction steps in the \( \text{H}_2 \)-oxidizing reaction of hydrogenase. One autocatalytic reaction occurs when the enzyme is mainly in inactive forms (Ni-A and/or Ni-B). This finding is supported by the special patterns of \( \text{H}_2 \) oxidation in a thin-layer reaction chamber and by the special, long lag phase observed. The autocatalytic step takes place between two enzyme forms, one of which also interacts directly with the terminal electron acceptor, i.e. the autocatalyst is an enzyme form in which [FeS] distal is reduced. During the autocatalytic interaction, an electron is passed from the autocatalyst to the other enzyme form, i.e. inactive hydrogenase serves as a substrate in this autocatalytic step for another, activated hydrogenase molecule.

The most obvious sign of this autocatalytic step is that in a thin layer reaction chamber the reaction starts from distinct points, spreading out continuously until the whole mixture becomes blue. New spots may appear and grow during the reaction. The spots eventually collide and fuse. Within experimental error, the spots grow at constant velocity during the experiment (Fig. 1). The reaction-diffusion front is direct evidence of an autocatalytic reaction.

The second autocatalytic step is located in the enzyme cycle. Its presence is supported by the facts that the Ni-R form is exclusively diamagnetic and the steady-state concentration of the product of the \( \text{H}_2 \)-oxidizing reaction (i.e. reduced benzyl viologen) displays a strong enzyme concentration dependence. This enzyme concentration dependence does not
depend on the “active-inactive” states of the enzyme, and remains valid in consecutive H₂-oxidizing - proton-reducing reaction series performed on the same sample (Fig. 2). This autocatalytic step involves an enzyme-enzyme interaction; both interacting enzyme forms should participate in the catalytic cycle of the enzyme. Since the reduction of all [FeS] clusters would also be possible in a non-autocatalytic reaction, we hypothesize a small conformational change in the enzyme, catalyzed by the autocatalyst, which removes a block in the electron flow in either the [NiFe]/[FeS]proximal or the [FeS]proximal/[FeS]distal reaction step, or removes a blockade of the penetration of gaseous H₂ from the surface to the [NiFe] cluster.

A number of autocatalytic enzyme reactions are known. In the peroxidase system, the inorganic reaction mechanism includes autocatalysis; the enzyme merely catalyzes certain of the reaction steps. In autophosphorylation, the inactive enzyme is the substrate of its active form, which transforms the inactive form into an active one. This is very similar to the first type of autocatalytic reaction in hydrogenase. Furthermore, in various allosteric enzyme reactions, the enzyme is simply activated/inhibited by its substrates or products.

In the second case, when the autocatalytic reaction occurs in the enzyme cycle, hydrogenase participates in a unique type of autocatalysis, where the autocatalyst enzyme form interacts with another enzyme form, resulting in a probably small conformational change in the enzyme-substrate. This scheme is very similar to the prion reactions, but significantly different from the other known autocatalytic enzyme reactions described above. As far as we are aware, such a phenomenon has not yet been observed experimentally previously for an enzyme. The main characteristic of this kind of enzyme reaction that there is no steady state kinetics in the enzyme reaction, i.e. the concentrations of different conformers in the enzyme catalytic cycle cannot be assumed to be constants ever.

RELEVANT RESEARCH IN THE HOST LABORATORY

Although there were several indications in the literature, our laboratory was pioneering in discovery of the autocatalytic reaction in the hydrogenase reaction cycle. We have identified the autocatalyst as an enzyme form in which the [FeS]distal is reduced. Recently we are trying to identify the exact enzyme form and the reaction steps where the autocatalytic reaction takes place. Preliminary temperature dependent activity and spectroscopic measurements were performed, and deviations from the “normal” enzyme behavior were observed. Under specific conditions an oscillation can also be observed in the reaction but the concrete details of this phenomenon are not known. We also build mathematical models in order to investigate the enzyme reaction and evaluate and model the experimental results.

SPECIFIC AIMS

Detect the suspected conformational change during the autocatalytic reaction by different spectroscopic methods. Experimentally clarify the parameters resulting oscillatory phenomena in hydrogenase reaction.
Find a mathematical model for the oscillations in the hydrogenase reactions. 
Find the proper experimental conditions where the different hydrogenase conformations can be crystallized.

Time course of H₂ oxidation in the modified thin-layer reaction chamber. Since the time courses in the experiment exhibited different lag phases, in this Figure the graphs have been shifted to uniform lag phase (t=0). Experimental conditions: enzyme concentration varying from 4 nM to 1060 nM, oxidized benzyl viologen concentration 400 µM.

MATERIAL AND METHODS

Significant publications

Representative recent research grants
“Investigation of the autocatalytic enzyme reaction of hydrogenase” (OTKA, 2005-2009)
“The oscillating hydrogenase reaction” (OTKA, 2011-2016)

Some of the latest students in the laboratory
Ősz J, Ph.D., 1999-2005; “The autocatalytic reaction of hydrogenase”
Branca RMM, Ph.D., 2002-2008; “Structure and function of a novel cytochrome c4 from the purple photosynthetic bacterium Thiochrista roseopersicina”
Pankotai-Bodó G, Ph.D., 2001-2009; “Investigation of the autocatalytic reaction of hydrogenase”
Bankó S, Ph.D., 2009-recent; “To be announced”
Janovics Zs, Ph.D., 2009-recent; “To be announced”

SUGGESTED READINGS