Autocatalyst form of the hydrogenase falls out from the enzyme cycle resulting different apparent saturation values of the product

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Introduction

Hydrogenases are metalloenzymes which catalyze the reversible oxidation of the molecular hydrogen $H_2 \rightleftharpoons 2H^+ + 2e^-$.
The membrane bound [NiFe] HynSL hydrogenase from purple photosynthetic bacteria *Thiocapsa roseopersicina* has high stability against oxygen, heat and proteolytic digestion which properties hydrogenases do not have in general. It consist of a small (~34 kDa) and a large (~64 kDa) subunit.
We recently demonstrated in the case of *T. roseopersicina* hydrogenase that the hydrogenase-catalyzed oxidation of hydrogen includes at least one autocatalytic step. This finding was based on the special patterns of the hydrogen-oxidation reaction in a thin-layer reaction chamber; on the autocatalytic oscillations in the fast absorption kinetics of the reduced benzyl viologen-initiated reaction of hydrogenase, and on the special and long lag phase observed in the hydrogen-oxidation experiments. We also proved that the autocatalytic step takes place between two enzyme forms, one of which is also directly interacting with the terminal electron acceptor (1-4).

Methods

For thin-layer experiments, we constructed a special reaction chamber, comprising a glass plate into which a cylindrical pit, 0.53 mm deep, and 36 mm in diameter was polished (Fig. 1 A) Addition of reaction mixture (400 ml) into the pit resulted in a uniform, plane parallel, 0.4 mm thick reaction layer and the reaction chamber can be illuminated from below. The reaction chamber was placed into a small anaerobic box with Plexiglas windows at the top and bottom (Fig. 1 B), with a septum in the top window. The chamber was flushed continuously with gaseous hydrogen; the surplus of hydrogen was released through a thin needle in the septum at the top of the anaerobic box (4).
We have applied a prism at a fixed position on the top of the chamber. The light collected by the prism was introduced through an optical fiber into a spectrometer. The transmission spectrum of the penetrating light through the sample was recorded in the range of 200-1100 nm using the SpectraSuite software. The collection time was 1 s. The measurements were performed at room temperature (23 ºC). The measurements were continued while the measured transmission values did not change in the visible spectral range.
The measurements were done depending on the enzyme concentration (1.6 nM-1.07 mM) and on the substrate concentration (benzyl viologen 0.1- 10 mM).

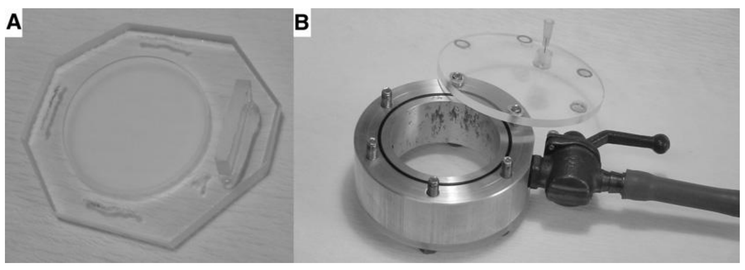

Fig. 1

Results

The reaction of hydrogenase in hydrogen oxidation direction was followed. With the prism and the connected detection system we were able to quantitatively follow the production of reduced benzyl viologen. Typical time courses of production of reduced benzyl viologen are presented in Fig. 2. It is clearly visible that higher enzyme concentration resulted in higher level of the steady state.
The dependence of apparent saturation values, derived from the steady state of the time courses, on enzyme concentration in the reaction volume is presented in Fig. 3 at different substrate (oxidized benzyl viologen) concentrations. The final optical density of the reaction mixture depended on both the enzyme and substrate concentrations, although at high enzyme concentrations it reached a saturation value.
At different substrate (benzyl viologen) concentrations the enzyme was able to reduce different ratio of the available substrate. More enzymes reduced higher proportion of the substrate and lowering the substrate concentration also increased the reduced ratio of the substrate in the steady state at the same enzyme concentration (Fig. 4).
The enzyme activity at lower enzyme concentration values depends on the enzyme concentration as a square root function (Fig. 5). It has already been previously observed this experiment confirms and supports that early observation.

Acknowledgment

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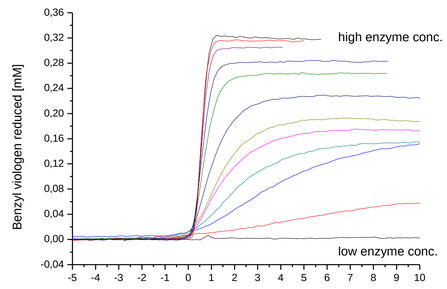

Fig. 2. Time course of the hydrogen oxidation reaction in the modified thin layer reaction chamber. Though time courses in the experiment have different lag phases, in this Figure the graphs were shifted to uniform lag phase (t=0). Experimental conditions: enzyme concentration is changing from 4 nM to 1060 nM, benzyl viologen concentration 400 µM.

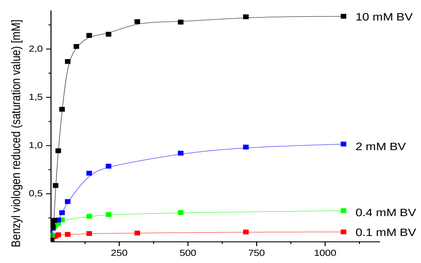

Fig. 3. Dependence of apparent saturation values determined from the time courses on enzyme concentration at different electron acceptor concentrations.

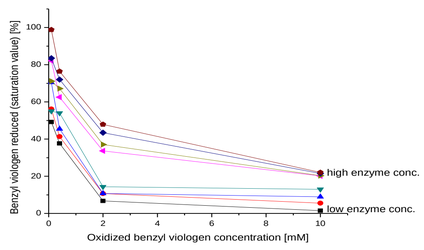

Fig. 4. Dependence of the apparent saturation value as a percentage of added oxidized electron acceptor concentration on added oxidized electron acceptor concentration at different enzyme concentrations. With increasing oxidized electron acceptor concentrations smaller and smaller proportion of the electron acceptor gets reduced. Increasing the enzyme concentration increases the final reduced proportion.

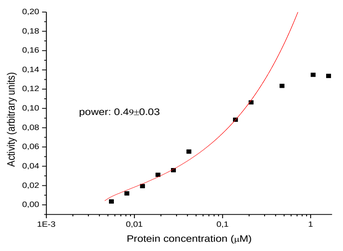

Fig. 5. Activity of the hydrogenase enzyme in thin layer reaction chamber (■) as determined from the time course (Fig. 1). The activity-enzyme concentration dependence function at lower enzyme concentration values follows the square root function (continuous curve) while at higher enzyme concentrations deviates from it. There is a threshold value in the enzyme concentration below which there is no activity at all. In this experiment it was estimated as 0.0036 µM.

Model calculations

The model calculations are based on stochastic calculations which are used the previously defined autocatalytic model (3). Classical model calculations were performed with slight modification. The saturation values calculated this way depending on the total enzyme concentration are presented in Figure 6.
Typical time courses of the process simulated in stochastic calculations are shown in Figure 7. Instead of concentrations, this figure shows the expectation value for the number of molecules of reduced benzyl viologen. Expectation values play approximately the same role as concentrations do in deterministic calculations. The different curves in Fig. 7 represent different numbers of enzyme molecules in the reaction while the other conditions are identical.
The stochastic model clearly predicts that the amount of product formed (reduced benzyl viologen) is rather limited by the amount of catalyst available then by the initial amounts of reactants present (H_2 and oxidized benzyl viologen). The distinct rise in the final expectation number of product molecules with increasing number of enzyme molecules is qualitatively the same to those seen in Figures 2 and 3; the stochastic model interprets the experimental data on a qualitative level. The fact that the autocatalytic step is within the catalytic cycle plays a key role in the model as this is the only feature facilitating the model to behave similarly to the experiment. All other model calculations failed to show this dependence.

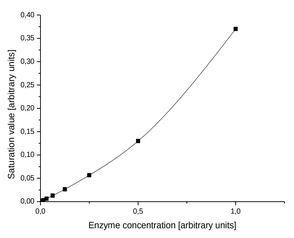

Fig. 6. Saturation values calculated by classical model calculations. Concentrations below 1 molecula/reaction volume have been substituted by exact zero..

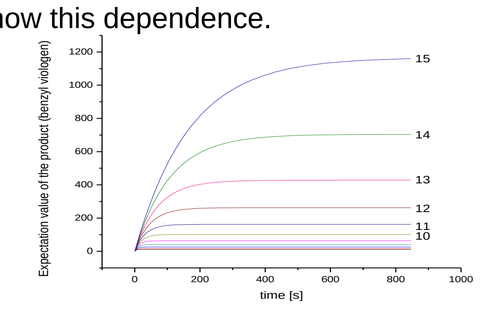

Fig. 7. Time course of the number of reduced electron akceptors at different total number of enzyme (5-15) as determined by stochastic calculation.

Conclusions

We have shown that the steady state of the enzyme reaction (the apparent saturation level) shows enzyme concentration dependence. This fact cannot be explained by a simple, Michaelis-Menten like enzyme cycle; it is an unequivocal proof of an autocatalytic enzyme step inside the enzyme catalytic cycle. A reaction model where there is an autocatalytic step included into the enzyme cycle qualitatively correctly describes the observed phenomena. There are, however, still some questions remained unanswered. The classical model calculation does not quantitatively depict the real experimental situation while the stochastic calculation cannot reach the experimentally evaluable region. Using the hydrogenase reaction model described in our earlier publications (1-4) we could not demonstrate the almost 100% conversion at 0.1 mM benzyl viologen and high enzyme concentrations. The model is also not answering why the conversion ratio reaches saturation on the enzyme concentration dependence. It definitely means that further refinement of the model is necessary.

References

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