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Oscillating hydrogenase reaction

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ABSTRACT

The hydrogenase-catalyzed oxidation of H_2 includes an autocatalytic step in the reaction cycle. The reaction also exhibits different pH dependence in the H_2 oxidation and in the proton reduction directions. This is not only due to the pH titration of the amino acid side chains as protons are also either the substrates or the products of the reaction. Utilizing the autocatalytic nature of the hydrogenase reaction and the multiple roles of protons therein, together with appropriate limitation of the substrate (gaseous H_2) supply, oscillations can be induced in the system. The reaction oscillates both in space and in time, and can last for days with decreasing frequency until reaching chemical equilibrium. Of all biological oscillating systems described so far, this one is the simplest in that it has the fewest biological components.

Introduction

Oscillating reactions are the basis of life: a great number of biological oscillations have been identified at the level of whole organisms, within individual living cells, as well as at molecular level [1]. The best studied are glycolytic oscillations which involve several enzymes and substrates [2,3], and the "peroxidase oscillation", where certain of the reaction steps are catalyzed by the enzyme peroxidase [4]. Also a well-known theme in biology is the circadian cycle, synchronized to the light–dark periods of the Earth [5–8]. The present paper reports on a much simpler biological oscillating system: it consists of a pure enzyme, the hydrogenase, together with its substrates and products.

Hydrogenases are metalloenzymes that catalyze the very simple reaction

$$H_2 \rightleftharpoons 2H^+ + 2e^-.$$

Protons, besides being substrates/products of this reaction, affect the enzyme activity as well. The reaction exhibits pH dependence; the maximum for H_2 production by *Thiocapsa roseopersicina* [NiFe] hydrogenase (HynSL) is at pH = 4, while that for H_2 uptake is at pH = 9.5 [9]. At physiological pH = 7, the two activities are very similar [9].

We recently demonstrated that the hydrogenase-catalyzed oxidation of H_2 (H_2 uptake) by *T. roseopersicina hynSL* [NiFe] hydrogenase includes at least one autocatalytic step (for the

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"canonic" enzyme reaction cycles and activation of the enzyme, see Refs. [10,11]). This finding was based on special patterns of the H_2-oxidation reaction in a thin-layer reaction chamber; the autocatalytic oscillations in the fast absorption kinetics of the reduced methyl viologen-initiated reaction of hydrogenase, and the special, long lag phase observed. We further proved that the autocatalytic step takes place between two enzyme forms, one of which also interacts directly with the terminal electron acceptor [15]. Such an autocatalytic reaction has also been demonstrated in the case of *Desulfovibrio baculatus* hydrogenase (A.N. Zorin and Cs. Bagyinka, unpublished) and therefore we think it is common at least in the [NiFe] hydrogenase family. This autocatalytic enzyme reaction is unique in the sense that both the autocatalyst and its substrate are different forms of the same enzyme, i.e. a protein–protein interaction happens during the autocatalytic step, resulting in a presumably small conformational change in the enzyme-substrate complex [12–15]. A similar protein–protein interaction was also suggested for prion proteins [16]. On the other hand, the reaction is unique because the autocatalytic reaction takes place in the enzyme cycle [12–15]. This is not the case for any other autocatalytic enzyme reactions.

Materials and methods

Purification of hydrogenase

The stable [NiFe] hydrogenase from *T. roseopersicina* (HynSL) was purified as described previously [12]. Both partially (before preparative gel electrophoresis) and fully purified enzymes were used for measurements, with no apparent difference in the results.

Thin-layer experiments

For thin-layer experiments, the same experimental setup was used as described in Ref. [15].

Sample preparation

The reaction mixture contained HynSL hydrogenase at a concentration of 200 nM in distilled water, and benzyl viologen (Sigma, St. Louis, MO) as artificial electron acceptor at a concentration of 2 mM. The chamber was flushed with gaseous N_2 for 10 min, and then it was closed. The reaction was started by injection of gaseous H_2. The initial concentration of the gaseous H_2 in the atmosphere was 5–10%.

Results and discussion

Product oscillation in the hydrogenase reaction is observed if the reaction mixture is not buffered (proton concentration is not fixed) and the initial gaseous H_2 concentration in the atmosphere is limited (i.e. it is initially set to ~5–10% and no further H_2 supply is maintained). The oscillation is followed by observing the color (oxidation state) change of the electron acceptor (methyl or benzyl viologen).

Oscillation appears throughout the whole reaction volume, as illustrated in the recorded movie (Supporting Online Material – 1). The oscillation can also be followed by measuring the optical density (Fig. 1). The oscillation has a long period, which increases considerably further as time passes. For this, and for technical reasons, we were able to record only two periods both in the recorded movie and by measuring the optical density. Data acquisition has been finished after several hours, but visual observation indicated that the reaction continued to oscillate over several days with longer and longer periods (data not shown). Stirring the reaction mixture affected neither the existence of oscillation nor the oscillatory characteristics. Without stirring, however, convection, too, plays a role in the spatial distribution of the reaction.

Supplementary video related to this article can be found at http://dx.doi.org/10.1016/j.ijhydene.2014.02.015.

When the reaction is carried out in a thin-layer reaction chamber, it can be demonstrated that the reaction oscillates both in space and in time. The reaction starts similarly to the buffered reaction [14,15], i.e. blue spots of the reduced electron acceptor (benzyl viologen) appear at different positions in the originally colorless reaction volume and the spots expand with constant velocity (Fig. 2 left panel). A colorless spot then appears in the centre of the blue spots, indicating the occurrence of the reverse reaction in the middle of the reaction circle (Fig. 2 right panel). The colorless spots then increase similarly to the blue spots at the beginning of the reaction.

The further evolution of the process in thin layer is apparently stochastic. The reaction volume sometimes

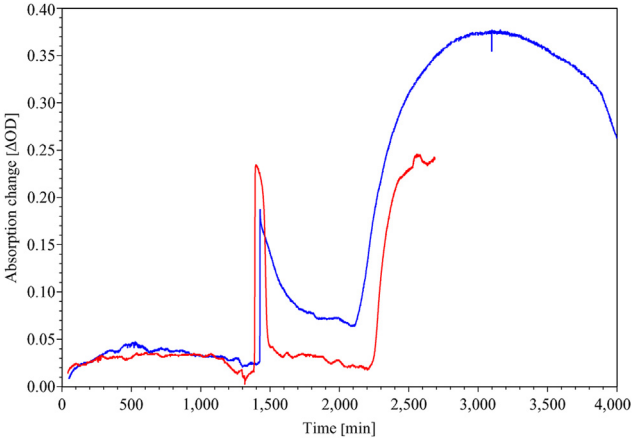

Fig. 1 – Bulk oscillation of hydrogenase. Absorption change of an unbuffered hydrogenase solution containing 2 mM benzyl viologen and 200 nM hydrogenase, with 50 μl (blue line) or 100 μl (red line) of gaseous H_2 added at the beginning of the reaction. The measurement was performed in an anaerobic cell with a path-length of 0.6 mm. After addition of H_2, the cell was vigorously shaken in order to distribute the H_2 evenly in the solution. Stirring the reaction did not affect the oscillation behavior of the reaction: stirred (blue line) and non-stirred (red line) reactions both exhibited oscillations. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)