Temperature dependence of the activity of hydrogenase from *Thiocapsa roseopersicina*

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1.

Introduction

Hydrogenases are metalloenzymes which catalyze the reversible reduction and oxidation of molecular hydrogen [1]:

$$H_2 \rightleftharpoons 2p^+ + 2e^-$$

Our previous results demonstrate an autocatalytic mechanism during the reaction cycle of HynSL hydrogenase from *Thiocapsa roseopersicina* [2,3]. This assumption was based on the special patterns of the hydrogenase-uptake reaction in a thin-layer reaction chamber. The membrane bound (HynSL) hydrogenase from this purple photosynthetic bacteria has high stability against oxygen, proteolytic digestion and heat which properties hydrogenases do not have in general [4]. We studied the temperature dependence of both hydrogen oxidation and hydrogen evolution activity of HynSL hydrogenase.

2.

Hydrogen uptake reaction

The reaction mixture contained enzyme in sodium phosphate pH 7.5 buffer and 50 mM benzyl viologen as artificial electron acceptor. The reaction mixture was distributed on the surface of a glass dish composing a 0.5 mm thin layer. The dish was placed in a Plexiglass anaerobic box, reaction was started by flushing the box with hydrogen gas and the color change of reducing benzyl viologen was followed by a video camera. Effect of temperature on the enzyme activity is studied from 10 to 50 ◦C using a thermostatic bath.

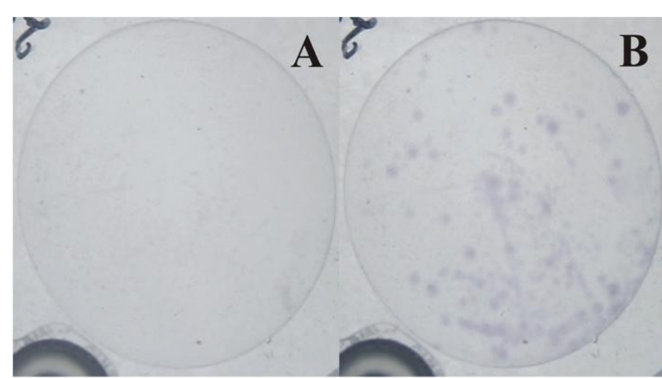

Figure 1. Hydrogen uptake reaction of hydrogenase below 30 ◦C shows a special spatial pattern in thin layer reaction mixture due to the autocatalytic nature of the enzyme. The reaction starts from distinct points which are expanding continuously (A, B) until the whole reaction mixture becomes blue.

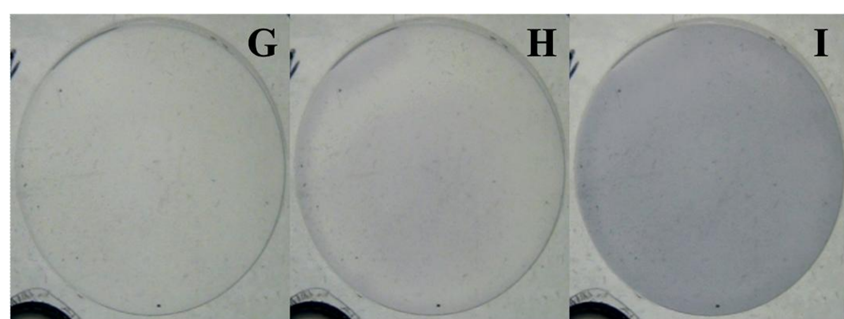

Figure 2. At higher temperature starting points could not be observed, the reaction mixture went blue homogeneously (G-I).

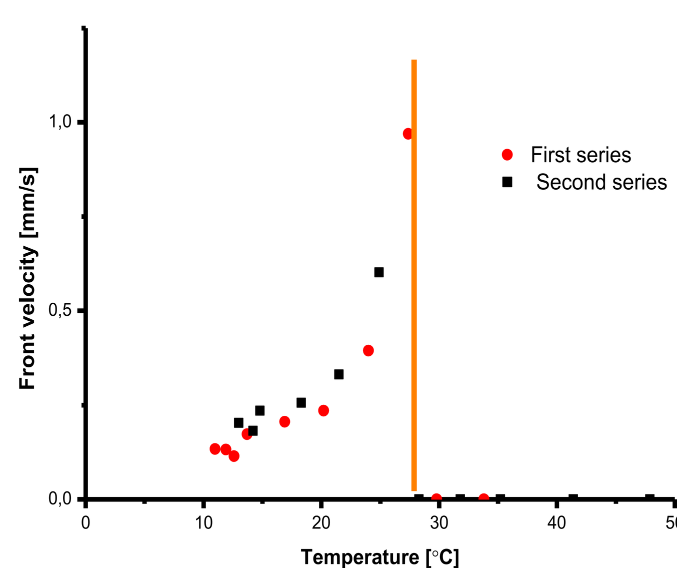

Figure 3. Above 27 ºC the autocatalytic reaction disappears from the process.

3.

Hydrogen evolution activity

The reaction mixture contained hydrogenase enzyme in 20 mM sodium phosphate pH 7.5 buffer and 2 mM methyl viologen (reduced by 10 mM dithionite) as artificial electron donor. The reaction mixture was placed in an anaerobic vial. The vial was flushed with nitrogen. The reaction was started by adding dithionite and the vials were incubated at different temperatures from 5 to 85 ◦C with 5 ◦C intervals as well as at 3, 26, 28, 33 ◦C using a thermostatic bath. The amount of evolved hydrogen was measured by a Thermo Scientific FOCUS gas chromatograph.

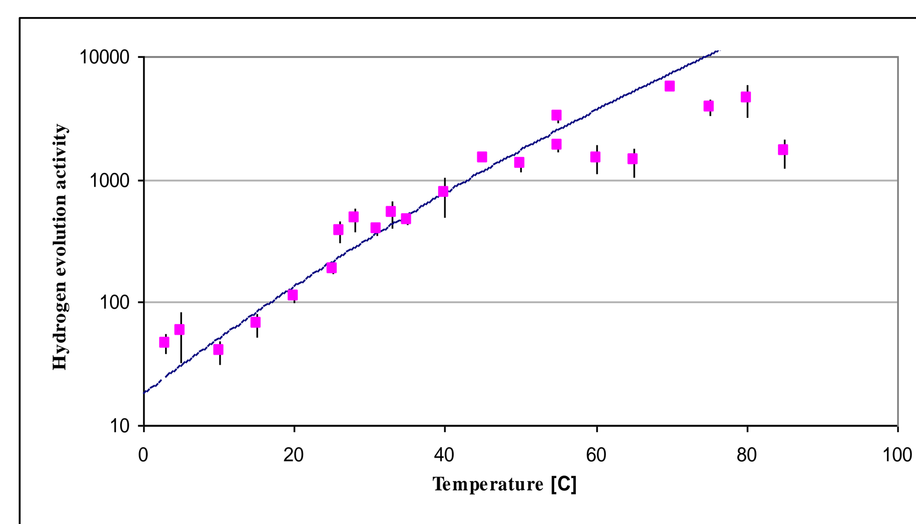

Figure 4. Effects of different temperatures on the hydrogen evolution activity of HynSL.

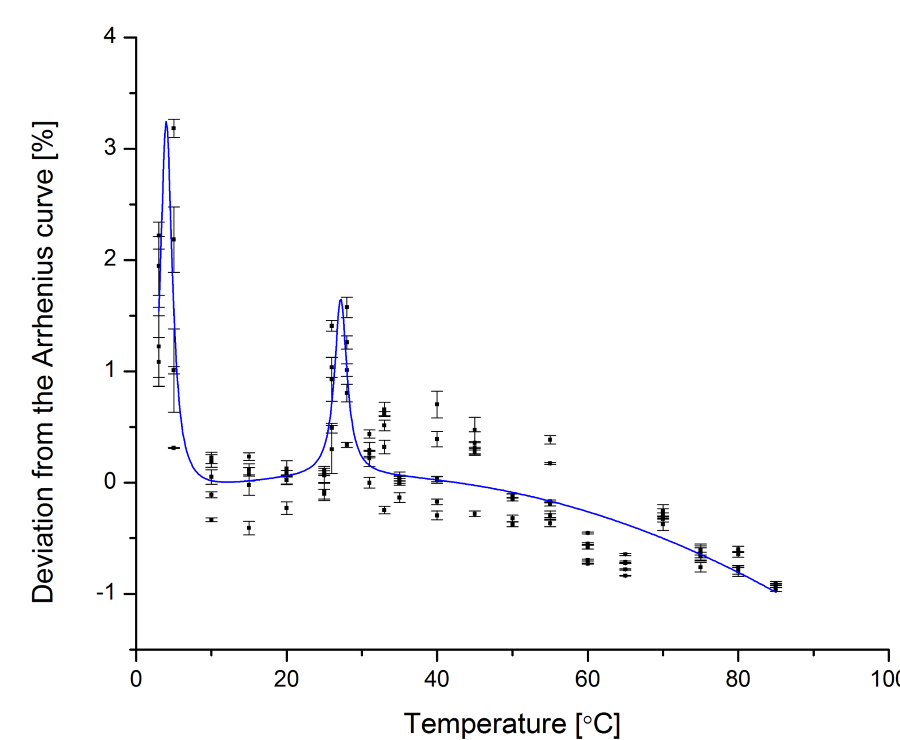

Figure 5. Relative deviation of the H_2 production activity from the Arrhenius curve.

4.

CD measurements

CD spectra were taken in a JASCO J-715 dichrograph equipped with a temperature-controlled sample holder. The sample holder was cooled/heated with a bench top thermostat, the samples were equilibrated at each temperature for 5 min. Overall, including the 5 min measuring time, the average heating rate was 0.5 ºC min^{-1}. The temperature dependences were measured in the far-UV range (190-250 nm) in a 0.1 mm quartz cell, and the average of five scans was collected. All spectra were recorded in absorbance units with a resolution of 1 nm and an integration time of 1 s. The data were analyzed by SVD analysis and the phase transitions were determined by fitting exponential jump functions to data SVD **v** vectors.

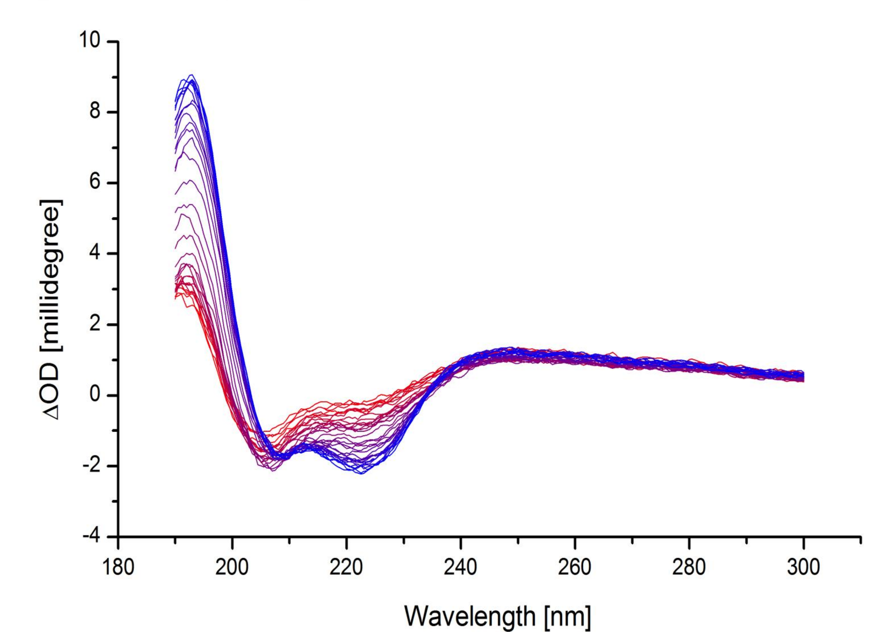

Figure 6. Temperature dependent CD spectra of oxidized hydrogenase. Blue curve represents the lowest temperature (0 °C) while the red curve represents the highest temperature (100 °C). The color transition represents temperatures between the two extreme values.

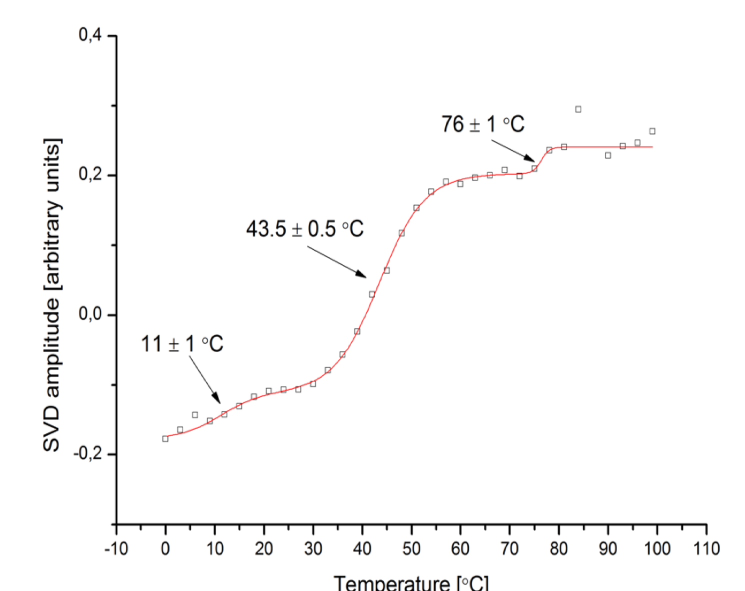

Figure 7. Phase transitions as determined from the SVD analysis of the CD spectra.

5.

Conclusions

- A new phenomenon, a singularity was observed in the autocatalytic reaction. Below 27 ºC the autocatalytic reaction was present and the front velocity was increasing by the temperature, but above 27 ºC the autocatalytic reaction disappeared in the process.
- Hydrogen evolution activity has shown an alteration from the Arrhenius dependence at this temperature, presenting a definite peak with the center of 25 ºC.
- Investigating the CD structure of the hydrogenase there were three phase transitions observed at 11, 45 and 72 ºC. None of them corresponded to the observed singularity; consequently it is probably caused by a small conformational change of the protein not observable by the CD experiments.

6.

References

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7.

Acknowledgments

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