CATALYTIC GENERATION OF HYDROGEN FROM ACIDIC AQUEOUS SOLUTIONS OF AQUA ION OF VANADIUM IN THE PRESENCE OF Thioecapsa roseopersicina HYDROGENASE AND METHYL VILOGEN

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Hydrogenases, which are among the enzymes of the oxidase-reductase class (EC 1.98.1.1), catalyze processes of release and absorption of molecular hydrogen in biological systems

\[ 2H^+ + 2e^- \rightarrow H_2 \]

and they are the best catalysts known at the present time for the release of \( H_2 \) from water under the influence of one-electron reducing agents. Research on the catalytic properties and mechanism of action of hydrogenases is very important for the development of new, effective, stable catalysts for \( H_2 \) generation.

It is known that hydrogenases are not highly specific and that they can release \( H_2 \) in the presence of various electron donors. Thus, in the case of Thioecapsa roseopersicina hydrogenase, the specific donors may be any one of a number of low-potential electron carriers, including flavodoxin, cytochrome \( c_1 \), and methyl and benzyl viologen [1], substances which differ in nature. Thus far, however, no data are available on the use of inorganic reducing agents as electron donors for hydrogenase.

Here we are reporting on a study of the possibility of using such a typical inorganic reducing agent as \( \text{V}^{5+}(\text{aq}) \) as an electron donor for \( \text{Th. roseopersicina} \) hydrogenase.

The \( \text{VOSO}_4 \) used in this work was obtained by reduction of an acidic solution of \( \text{VOSO}_4 \) on \( \text{Zn} \) amalgam in an \( \text{Ar} \) atmosphere. The reagents that were used were chemically pure grade and analytically pure grade. The recovery and purification of the hydrogenase from the cells of the purple sulfur bacterium \( \text{Th. roseopersicina} \) BB were performed by procedures given in [2]. The activities of the hydrogenase preparations were determined by gas chromatography on the basis of the quantity of \( H_2 \) released within a certain time in a static reactor in an \( \text{Ar} \) atmosphere, from a 2.10^{-6} M solution of methyl viologen \( \text{MeVi}^{2+} \) in 0.02 M phosphate buffer (pH 7.1) in the presence of \( 10^{-3} \text{M Na}_2\text{S}_2\text{O}_4 \), at 303 K. The activity of the original hydrogenase preparation was \( 4 \) \( \mu \)moles \( H_2/\text{min-ml} \) of hydrogenase, and was close to its standard activity \( \approx 100 \) \( \mu \)moles \( H_2/\text{min-mg} \) of hydrogenase [1].

The thermodynamic requirement placed on reducing agents used for generation of \( H_2 \) from water is that their reduction potential must be more negative in relation to the NHE, i.e., \( E_0 \leq -0.059 \cdot \text{pH} \) (in V, relative to NHE). Thus, \( \text{V}^{5+}(\text{aq}) \), with \( E_0 = -0.26 \text{V} \), in the presence of an appropriate catalyst, should release \( H_2 \) at \( \text{pH} \lesssim 4.5 \). However, hydrogenases are unstable in acidic media [1], even though very little specific information is available on the activity in the region of \( \text{pH} \approx 5 \).

We have investigated the stability of \( \text{Th. roseopersicina} \) hydrogenase in an acidic medium by incubation of the hydrogenase preparation for various times in acidic solutions, after which the enzyme activity was determined by the method described above. We found that when the preparations were incubated in air at \( \text{pH} 4.1 \) in biphthalate buffer solution for 30 min, the hydrogenase does not undergo deactivation; it is possible that there may even be a slight activation of the enzyme (Fig. 1). Upon incubation in air at \( \text{pH} 3 \), a hydrogen sulfide odor appears, and inactivation of the hydrogenase is observed, amounting to about 85% in 5 min and about 95% in 30 min. Under anaerobic conditions, the deactivation proceeds at approximately the same rate (see Fig. 1).
Fig. 1. Deactivation of Th. roseopersicina hydrogenase during incubation in air in buffer solution (K acid phthalate) at pH 4.1 (1), 2.9 (2), or 3.5 (3), and during incubation under anaerobic conditions at pH 3.5 (4). \( \sigma \) is the relative activity, calculated as the measured activity of the hydrogenase expressed as a percentage of the activity of the original hydrogenase preparation.

Fig. 2. Kinetics of \( \text{H}_2 \) evolution from 5 ml of solution at pH 4.5, containing hydrogenase, \( 10^{-2} \) M \( \text{V}^{2+} \), and \( 2 \times 10^{-4} \) M methyl viologen, at 303°K.

Thus, Th. roseopersicina hydrogenase at pH \( \geq 4 \) is stable for a comparatively long time (>0.5 h). In order to determine the feasibility of using \( \text{V}^{2+} \) (aq) as an electron donor for Th. roseopersicina hydrogenase, we introduced 50 \( \mu l \) of the hydrogenase preparation into 5 ml of \( 10^{-2} \) M solution of \( \text{V}^{2+} \) at 303°K and pH 4.5, established by adding \( \text{H}_2\text{SO}_4 \) (Fig. 2). Here we did not observe any \( \text{H}_2 \) evolution, within the accuracy of the experiment (i.e., \( \approx 10^{-6} \) mole \( \text{H}_2 \) in 10 min).

The addition of freshly deoxygenated \( \text{MeV}^{2+} \) solution to the reaction mixture (to a concentration of \( 2 \times 10^{-4} \) M) leads to the appearance of a bright blue color, characteristic for the cation radical of methyl viologen. Also observed is rapid evolution of \( \text{H}_2 \) at the rate characteristic for this particular hydrogenase preparation at 303°K and pH 7 in the presence of \( 2 \times 10^{-4} \) M \( \text{MeV}^{2+} \) and excess \( \text{Na}_2\text{S}_2\text{O}_5 \). The total quantity of hydrogen released is approximately 4 times the quantity of \( \text{MeV}^{2+} \) added. Since no hydrogen is evolved from solutions of \( \text{V}^{2+} \) and \( \text{MeV}^{2+} \) in the absence of the hydrogenase, we should conclude that the \( \text{MeV}^{2+} \) cations play the role of a carrier of electrons between the \( \text{V}^{2+} \) and the hydrogenase, the reaction of electron transfer from the \( \text{V}^{2+} \) to the \( \text{MeV}^{2+} \) not limiting the process of \( \text{H}_2 \) evolution. The incapability of the \( \text{V}^{2+} \) cation for transferring an electron directly to the hydrogenase can be explained by the fact that it does not have any hydrophobic section, which may be necessary for specific interaction with the enzyme.

It is interesting to note that the standard electrochemical reduction potential of the \( \text{MeV}^{+} \) is more negative (\( -0.44 \) V relative to NHE) than for the \( \text{V}^{2+} (-0.26 \) V); i.e., the process of reduction of the methyl viologen by the \( \text{V}^{2+} \) cations proceeds in opposition to the gradient of the standard redox potential. However, we cannot eliminate the possibility that, in actuality, as a result of significant hydrolysis of the \( \text{V}^{2+} \) ions at pH 4.5, the reducing potential of the \( \text{V}^{2+} \) under the conditions we have described may be somewhat more negative than indicated by the value of the standard potential.

CONCLUSIONS

1. It has been shown that Th. roseopersicina hydrogenase can function in an acidic medium (pH 4.5) without any loss of activity over the course of \( \geq 0.5 \) h.

2. Feasibility has been demonstrated for the use of vanadium(II) aqua ions as an electron donor for Th. roseopersicina hydrogenase in the presence of small quantities of methyl viologen, used as an intermediate electron carrier.

LITERATURE CITED