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Short communication

Direct and electrically wired bioelectrocatalysis by hydrogenase from *Thiocapsa roseopersicina*

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Abstract

Hydrogen enzyme electrodes based on direct and mediated bioelectrocatalysis were developed. Direct bioelectrocatalysis of hydrogen oxidation/evolution was observed for hydrogenase adsorbed on carbon filament material. The equilibrium hydrogen potential was achieved on mediatorless hydrogen enzyme electrodes in hydrogen atmosphere. The electrocatalytic activity of hydrogenase in direct bioelectrocatalysis of hydrogen oxidation was two orders of magnitude higher compared to platinum. The reported electrode remained 50% activity after 6 months of storage with periodical testing. Wired bioelectrocatalysis was achieved by adsorption of hydrogenase onto electropolymerized redox mediator *N*-methyl-*N*′ -(12-pyrrol-1-yl-dodecyl)-4,4′ -bipyridinium ditetrafluoroborate.

Keywords: Hydrogenase; Direct bioelectrocatalysis; Enzyme electrodes; Wired enzyme; Polypyrrole

1. Introduction

The enzymes responsible in nature for oxidation/evolution of molecular hydrogen are trivially called ‘hydrogenases’ [1]. The hydrogenase enzyme electrode uses the catalytic properties of the hydrogenase enzyme able to activate hydrogen molecule for energy interconversion between H_2 and electricity. The development of enzyme electrodes requires the successful coupling of the enzyme with the electrochemical reactions.

The most attractive coupling is a direct electron exchange between the electrode and the active site of the enzyme.

The phenomenon of direct bioelectrocatalysis by hydrogenases was shown in the beginning of the 1980s [2,3]. In particular, hydrogenase of *Thiocapsa roseopersicina* being adsorbed on carbon black electrodes catalyzed electrochemical oxidation–evolution of hydrogen in the absence of any diffusion-free or immobilized mediators [2]. However, the modification of electrode surfaces by redox mediators in order to establish an electrical communication with enzyme molecules remains an important problem in the field of enzyme electrodes [4].

In current research, we have been studying both approaches for hydrogenase electrodes development.

Since the previous investigation of hydrogenase electrochemistry was carried out using carbon black modified electrodes, it was decided to immobilize the enzyme onto the commercial carbon materials. On one hand, the commercial carbon materials are accessible and well characterized, what provides the reproducibility of our results on hydrogen enzyme electrode. On the other hand, the use of commercial carbon supports is required for further commercial applications.

For direct bioelectrocatalysis studies, the hydrogenase was adsorbed onto the carbon filament material (CFM) specially designed by the Russian State company ‘Alten’ for development of fuel cells.

2. Experimental

Galvanostatic and voltammetric studies were performed using Solartron Schlumberger Model 1286 (UK). Experi-

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ments were carried out in a three-compartment electrochemical cell containing a Pt net auxiliary electrode and a reversible hydrogen reference electrode under constant hydrogen flow.

Glassy carbon disk electrodes (diameter 1.8 mm) made from glassy carbon type GC 2500 produced in Research Institute for Graphite Materials (Moscow, Russia) and carbon filament material CFM electrodes (1×0.5 cm) (TVS-300 M, Alten, Moscow Region, Russia) were used as working electrodes. CFM specific electrical resistance was 50–70 mΩ cm.

The hydrogenase from *T. roseopersicina* strain BBS was purified according to procedure described in Ref. [5] to 90 % of purity and characterized by activity of 20–40 μmol (H_2)/min per mg of protein.

Hydrogenase electrode type I was prepared by enzyme adsorption from its aqueous solution (2–4 mg/ml) for 12 h at 4 °C in 0.005 M K-phosphate buffer, pH 7.0 onto CFM electrodes.

The solution of Compound I (CI: *N*-methyl-*N*′-(12-pyrrol-1-yl-dodecyl)-4,4′-bipyridinium ditetrafluoroborate) in CH_3CN (2 mM, 5 μl) was deposited on glassy carbon electrode and allowed to dry for 30 min in ambient conditions. Electropolymerization included cycling of the applied potential from −0.8 to +0.95 V vs. Ag/AgCl(1 M) reference electrode with the sweep rate 50 mV/s for 30 min in 0.1 M $LiClO_4$ solution pH 6.0.

Hydrogenase electrode type II was prepared by enzyme adsorption from its aqueous solution (2–4 mg/ml) for 12 h at 4 °C in 0.005 M K-phosphate buffer, pH 7.0 onto the electrodes modified with poly(CI).

3. Results and discussion

A procedure for hydrogen enzyme electrode type I preparation includes the pretreatment of electrode support

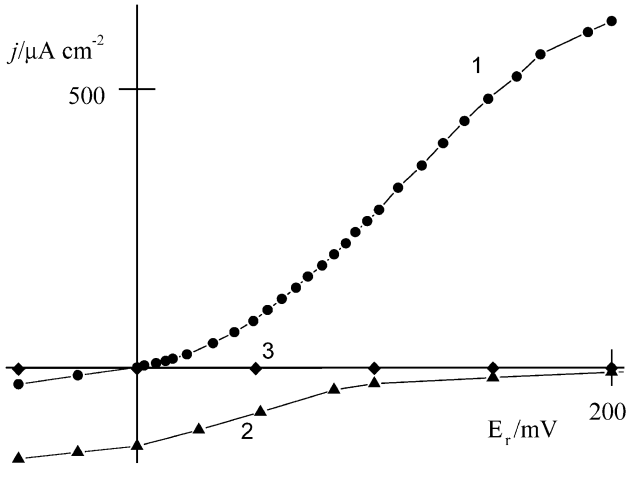

Fig. 1. Hydrogenase CFM electrode (type I) in H_2 (1), Ar (2) and blank electrode (3). Phosphate buffer pH = 7.0. Potential vs. H_2/Pt electrode in same buffer.

Table 1
Electrocatalytic properties of CFM hydrogenase electrodes

	j_0 (μA cm^{-2})	$10^{19} I_0$ per molecule of catalyst (A)
Hydrogenase electrode	11 ± 1	8 ± 1
Pt (pH 7.0)	< 10	< 0.1

followed by enzyme immobilization. Electrode pretreatment is a crucial step, especially taking into account, that initially CFM is completely hydrophobic. The optimal hydrophylization procedure included the treatment with sulfuric acid for 10–30 min followed by washing with buffer during 48 h.

After immersion of the enzyme electrode in neutral buffer solution saturated with molecular hydrogen, the equilibrium hydrogen potential has been achieved in less than 5 min. Hydrogen enzyme electrode was characterized by high values of anodic current at positive potentials (E_r) (Fig. 1). Without active hydrogenase the background reaction of hydrogen electrooxidation in the observed region of potentials is absent. In the absence of molecular hydrogen, the only cathodic current was observed for hydrogen enzyme electrode (Fig. 1). Thus, the anodic current is due to hydrogen oxidation on hydrogenase electrode, and the standard potential observed is the hydrogen equilibrium potential. Positive wave of the current–potential curve was not linearized in the traditional Tafel plots, but was fit to the two-exponential equation evaluated for the two sequential one-electron electrochemical stages.

$$j = 2\frac{\exp\left(2\frac{\alpha FE}{RT}\right) - \exp\left(-2\frac{(1-\alpha)FE}{RT}\right)}{\frac{1}{j_{o1}}\exp\left(\frac{\alpha FE}{RT}\right) + \frac{1}{j_{o2}}\exp\left(-\frac{(1-\alpha)FE}{RT}\right)} \quad (1)$$

where j_{o1} and j_{o2} are exchange current densities of first and second electrochemical stages, respectively. We let $\alpha = 0.5$ as for most electrochemical reactions.

The important characteristic of electrocatalyst is the exchange current (j_0) of the corresponding catalyzed reaction. This value can be found as from Tafel plots (if applicable for linearization), as from the j_{o1} and j_{o2} of the above equation. Exchange current density was taken as doubled of the lowest value of the j_{o1} and j_{o2}. Table 1 summarizes the values of the exchange current calculated as per electrode unit area as per molecule of the catalyst. It is seen that in neutral aqueous solutions, platinum electrode possesses similar electrocatalytic activity relatively the geometric electrode area. However, when recalculated per molecule of the catalyst, the efficiency of the enzyme becomes two orders of magnitude higher. The amount of adsorbed hydrogenase was estimated from reaction of hydrogen evolution in the presence of reduced methyl viologen according to Ref. [5]. The number of platinum atoms per unit area was calculated from value of equilibrium hydrogen adsorption (220 μC cm^{-2}) [6].

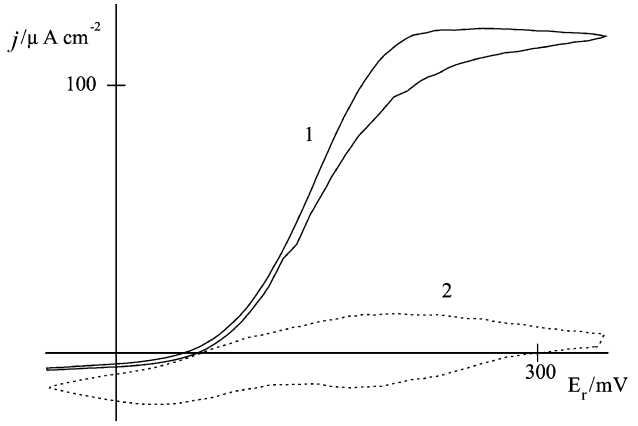

Fig. 2. Hydrogenase poly(CI) electrode (type II) in H_2 (1) and Ar (2). Potential sweep rate 2 mV/s. Phosphate buffer pH = 7.0. Potential vs. H_2/Pt electrode in same buffer.

Thus, the enzyme electrode based on carbon filament material is characterized by high efficiency: hydrogen equilibrium potential and high current of hydrogen oxidation. Before that hydrogen equilibrium potential was firstly registered for *T. roseopersicina* hydrogenase immobilized on gold covered with carbon black [2] or for *Desulfovibrio vulgaris* hydrogenase, but in the presence of mediators [7].

A crucial factor for biotechnology applications is the stability of the enzyme electrode. Hydrogenase immobilization onto the CFM causes the dramatic improvement of both operational and storage stability. Even after half a year of storage with periodical testing, the enzyme electrode type I preserved more than 50% of its initial activity.

To realize the wired bioelectrocatalysis by hydrogenase, we used electropolymerized redox mediators for the immobilization of hydrogenase. For this aim, the chemical synthesis of viologen substituted by an electropolymerizable group (pyrrole) and also a long alkyl chain based on 12 carbons (Compound I) has been made.

Methyl viologen is known as the most popular redox mediator used for biochemistry of hydrogenases [7,8]. The preliminary electrochemical experiments have demonstrated the possibility to electrogenerate electroactive polymers exhibiting the redox behavior of the viologen unit.

The possibility of an electrical wiring of hydrogenase was shown (Fig. 2). It is seen that in hydrogen atmosphere, the cyclic voltammogram of 'wired' hydrogenase electrode has a shape of catalytic reaction. At positive potentials (> 50 mV), enzyme electrode exhibited high values of positive current, whereas in the absence of H_2 the only background CV of electroactive polymer was observed.

The high anodic current, thus, peculiar to hydrogen oxidation reached high values, which taking into account the morphology of the electrode supports (filament fabric for direct bioelectrocatalysis and smooth surface for wired one) are nearly of similar order with the current obtained in case of direct bioelectrocatalysis. However, in case of 'wired' hydrogenase, we were not able to reach hydrogen equilibrium potential because the standard potential of the electropolymerized mediator was in the range 100–200 mV.

4. Conclusion

We conclude that both direct and 'wired' bioelectrocatalysis can be realized in case of hydrogenase enzyme electrodes to achieve high efficiency of hydrogen oxidation. For hydrogen evolution, it seems the enzymes from the other sources, which are tremendously more active in this reaction, have to be used.

Acknowledgements

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