Limiting performance characteristics in bioelectrocatalysis by hydrogenase enzymes

Arkady A. Karyakin*, Sergey V. Morozov, Oleg G. Voronin, Nikolay A. Zorin, Elena E. Karyakina, Vladimir N. Fateyev, Serge Cosnier

Materials

The solutions throughout this work were prepared using distilled water from a Milli-Q system (Millipore, Bedford, MA). All other reagents used were of analytical reagent grade. The hydrogen was produced by water electrolysis (gas supplying system SGS-2, Russia).

The [NiFe] hydrogenases from Thiocapsa roseopersicina strain BBS and Lamprobacter modestogallofillum were purified according to procedure described in [1,2], respectively, to 90 % of purity. Hydrogenase from Desulfomicrobium baculatum was purified as follows. Cell walls were destroyed by osmotic shock by two freezing-melting cycles in 50 mM Tris-buffer, pH 7.5 with subsequent centrifugation (20 min, 15000 g). Hydrogenase was purified by ion-exchange chromatography on DEAE-cellulose (8x2 cm, DE52 Whatman, UK) and phenyl-sepharose (20x1 cm, Phenyl-Sepharose CL-4B "Pharmacia", Sweden) with gradient of NaCl.

Hydrogenase electrodes preparation

Carbon filament material LSG (1x0.5cm) (electrical resistivity of 50-70 mΩ cm and surface roughness of 10-30, ‘Alten’ Company, Moscow Region, Russia) was used as working electrode.

Hydrogenase electrodes were 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 CFM electrodes modified with poly(MPDBP). The solution of (MPDBP) in CH3CN (2mM, 100 μl) was deposited on CFM electrode and allowed to dry for 2h in ambient conditions. Electrochemical polymerization was carried out by repeated potential cycling over the range −0.8 - +0.95 V vs. Ag/AgCl|1M KCl reference electrode with the sweep rate 50 mV/s for 40 min in 0.1 M LiClO4 solution pH=6.0. After polymerization the electrodes were washed with phosphate buffer solution (0.05M KH2PO4, 0.1M KCl, pH=7.0) for 10 minutes. The apparent surface coverage of the electopolymerized (MPDBP) (Γ≈1.4 10⁻⁷ mol cm⁻²) was determined from the charge recorded under the reversible peak system assigned to the one-electron reduction of the polymerized viologen group, leading to an electropolymerization yield of 39 %.

* Corresponding author
 Electrochemical characterization of hydrogenase electrodes

A three-compartment electrochemical cell contained a platinum net auxiliary electrode and a platinum black hydrogen electrode in the same solution as a reference (reversible hydrogen electrode RHE). The cell construction allowed deaeration of the working electrode compartment. Electrochemical activity of electrodes was tested in galvanostatic mode using Solartron Electrochemical Interface 1286 (UK) with constant gas flow through solution and stirring. All experiments were made in phosphate buffer solution (0.05M KH₂PO₄, 0.1M KCl, pH=7.0). The whole electrochemical cell was thermostated during experiments. The amount of oxygen in H₂ was monitored with gas analyzer PKG-4-K («Praktic-NC» Company, Moscow Region, Russia) connected to gas outlet.

Determination of catalytic constant in electrocatalysis ($k_{\text{cat}}$)

Rate of enzyme reaction is generally determined by Michaelis-Menten equation:

$$v = \frac{V_M [S]}{K_M + [S]}$$

where $V_M$ is the maximum rate, $K_M$ is Michaelis constant, $[S]$ is substrate concentration. $V_M$ is equal to $k_{\text{cat}}[E]_0$, where $k_{\text{cat}}$ is catalytic constant, $[E]_0$ is enzyme concentration.

In hydrogen saturated solutions both homogeneous and electrochemical kinetics of hydrogenase action is saturated with H₂ [4,5], see also figure at moderate overvoltages. Enzyme catalytic constants concerning homogeneous kinetics ($k_{\text{kin}}$) were found from kinetics of H₂ oxidation by methylviologen monitored spectrophotometrically. Plotting initial rates of hydrogen oxidation vs methylviologen concentration in different coordinates it was possible to determine $V_M$ and, hence, $k_{\text{cat}}$, which here is referred to as $k_{\text{kin}}$.

In bioelectrocatalysis, we have to consider both electrochemical stage (electron exchange between enzyme active site and the electrode) and enzyme catalytic stage, obviously occurred sequentially:

$$E + S \underset{\text{cat}}{\xrightarrow{\text{c}}} E' \underset{\text{e}}{\xrightarrow{\text{c}}} E + P$$

Catalytic step is always assumed to be irreversible, and electrochemical step at overvoltages > 50 mV is also irreversible. Our calculations were made at 200 mV overvoltage.

The constant for the whole reaction is known to obey the equation:

$$k_{\text{e/e}} = \left( \frac{1}{k_{\text{cat}}} + \frac{1}{k_e} \right)^{-1}$$

which introduces the conception of 'rate determining step' for sequential reactions: the rate of the whole reaction will be controlled by the slowest stage. Since electrochemical rate
constant is exponentially increased with overvoltage and catalytic constant is independent of the potential, catalytic stage at certain overvoltages determines the rate of the whole reaction. Hence, at large overvoltages we face the kinetic limiting process $k_{e/c} \approx k_{cat}$.

Now we transfer current density to reaction rate by

$$i = nFv$$

, where $F$ is Faraday constant and $n$ is the number of electrons transferred per catalytic cycle. The final equation for catalytic constant found from electrocatalysis is

$$k_{e/c} = \frac{i_{200}}{nF[E]}$$

, where $[E]$ is now surface enzyme concentration (enzyme loading).

Figure. Current-voltage curves of *T. roseopersicina* hydrogenase electrode under hydrogen-argon mixtures at different H\textsubscript{2} content.