BIOELECTROCATALYSIS. ACTIVATION OF A CATHODE OXYGEN REDUCTION IN THE PEROXIDASE-MEDIATOR CARBON ELECTRODE SYSTEM

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1. Introduction

At the present time effects are known that increase the rates of electrode processes in electrochemical systems in the presence of biological catalysts. Thus the rate of glucose oxidation increases in the presence of immobilized glucose oxidase [1,2]. With bacterial hydrogenases electrochemical hydrogen ionization may occur on carbon electrodes at a rate close to that observed in the presence of the 'classical' catalyst, metallic platinum [3]. Recently a range of such acceleration effects of the electrode processes with biocatalysts was established. The effect of bioelectrocatalysis is to increase the rate of an electrochemical reaction by the presence of enzymes. Interest in bioelectrocatalytical effects was considerably stimulated by the possibility of creating electrochemical transformers, that may oxidize different fuels and generate an electrochemical potential [3].

The main problem in the bioelectrocatalysis is the conjugation of enzymatic and electrochemical reaction steps. In general this may take place via the following two mechanisms:

(1) Electron transfer from the enzyme active centre to an electrode is performed by a mediator

$$S + E \longrightarrow P + E^{\circ}$$

$$E^{\circ} + M \longrightarrow E + M^{\circ}$$

$$M^{\circ} \xrightarrow{\text{electrode}} M \pm e^{-} \qquad (1)$$

where S is an oxidized (or reduced) compound, M,M° are oxidized and reduced mediator forms, and E,E° are oxidized and reduced forms of an enzyme active site.

(2) The direct electron transfer from an active site of enzyme to an electrode

$$S + E \longrightarrow P + E^{\circ}$$

$$E^{\circ} \xrightarrow{\text{electrode}} E \pm e^{-} \qquad (2)$$

Electron transfer via a mediator is obviously easier than the direct transfer of electrons from the enzyme active site to an electrode matrix. For example the system where this process was efficiently realised with participation of an hydrogenase, the enzymic electrochemical anodic oxidation of hydrogen being brought about [3]. The present study is concerned with development of a second electrode at which cathodic oxygen reduction occurs in the presence of an enzyme.

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Methods and materials

The electrochemical measurements were made using a rotating disc electrode of pyrographite with 0.2 sq.cm surface. The experimental technique has been described in [4]. A hydrogen electrode served as a reference, the electrochemical potentials being scaled to a hydrogen electrode in the same solution.

Horse radish peroxidase (Rz 0.6) from 'Reanal' was used without additional purification. Hydroquinone, manganese nitrate ($Mn(NO_3)_2.6H_2O$) and helium were thoroughly purified.

Results and discussion

We attempted to use a haem-containing enzyme, peroxidase, as a catalyst to activate oxygen reduction. The main function of the enzyme being to catalyze oxidation of organic compounds by hydrogen peroxide. Moreover, it is known that peroxidase possesses an oxidase activity i.e. an ability to catalyze substrate oxidation by molecular oxygen [5].

Preliminary experiments with peroxidase showed that it does not accelerate an electroreduction of oxygen on pyrographite in neutral solution. When the enzyme solution was saturated with oxygen and the polarization curves measured, the difference between reduction curves with and without the catalyst was negligible. These experiments show that there was no direct electron transport from the enzyme active centre to the electrode.

Hydroquinone was then used as an electron transfer molecule. First hydroquinone polarography on a pyrographite electrode was studied. The hydroquinone solution was bubbled with helium and the current–potential plot recorded in the range −0.5–+1.0 V. The results for the quinone–hydroquinone system are shown in fig.1. Curve 1 is for hydroquinone alone in solution. The redox potential of quinone–hydroquinone system has a value of 0.7 V. Curve 2 was measured after equilibrium between quinone and hydroquinone was achieved in the course of electrochemical hydroquinone oxidation. Thus the results obtained show the reversible pattern of electrochemical reactions of the quinone–hydroquinone pair on the pyrographite electrode.

An addition of hydroquinone to the peroxidase

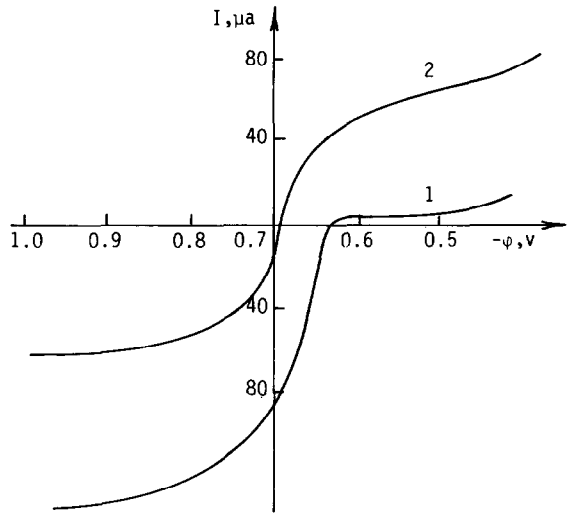


Fig.1. The anode–cathode polarization curves of hydroquinone (curve 1), of hydroquinone–quinone (curve 2) pH 7.2, 0.01 M of sodium phosphate, 0.1 M of potassium chloride, 25°C. The total hydroquinone plus quinone concentrations were always 1 mM. The experiments were carried out under helium.

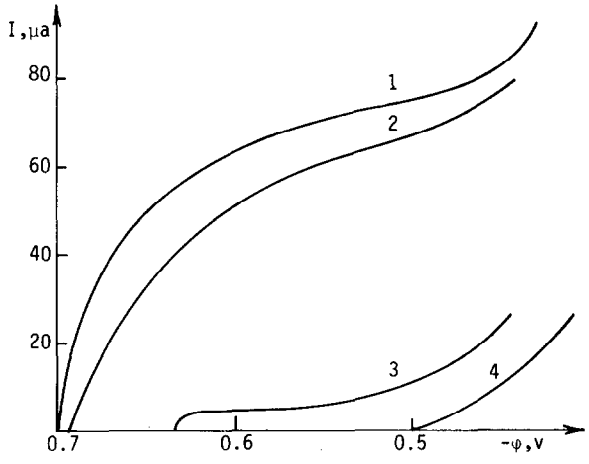
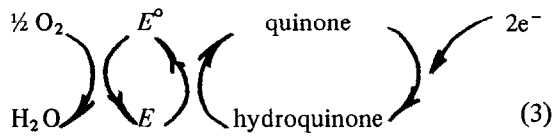


Fig.2. The cathode polarization curves of the oxygen–quinone–hydroquinone–peroxidase–pyrographite electrode system. Curve 1 with peroxidase (0.5 mg/ml) and manganese ions (0.1 mM). Curve 2 with peroxidase without manganese ions. Curve 3 the cathode reduction of quinone. Curve 4 the cathode reduction of oxygen, pH 7.2, 0.01 M of sodium phosphate, 0.1 M of potassium chloride, 25°C.

solution saturated with oxygen essentially enables the effective rate of the electrodic reaction to be increased. Figure 2 presents the cathode polarization curves of the system oxygen–peroxidase–quinone–pyrographite electrode (curve 1). Additional increase in the rate is attained by introduction of manganese ions (curve 2). It is known that manganese ions activate the oxidase activity of peroxidase. Figure 2 also gives, for comparison, the polarization curves of oxygen electroreduction on pyrographite without catalyst (curve 3) and quinone electroreduction without catalyst (curve 4).

Special experiments have demonstrated that hydroquinone oxidation with oxygen is also catalyzed by manganese ions but the hydroquinone oxidation rate is considerably higher when both peroxidase and manganese are present. Thus the presence of peroxidase activated with manganese ions and a mediator enable one to shift the effective reduction potential of the oxygen by about 300 mV to the positive region. The total reaction proceeding in the solution and at an electrode surface may be described by the scheme:



(3)

where E and E° are the oxidized and reduced forms of peroxidase active centre. In this system the quinone–hydroquinone active pair plays the role of a low molecular weight electron transporter between enzyme active centre and an electrode.

Scheme (3) is somewhat idealized since it takes no account of some complicating processes associated with mediator degradation and dimerization.

We thus succeeded in activating the cathode reduction of oxygen in the peroxidase-mediator pyrographite-electrode system. Important progress from both theoretical and practical points of view would be the development of direct electron transport between enzyme active centre and an electrode. We are now studying the possibility of realizing such a process.

Acknowledgement

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