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The mechanism of cathode reduction of oxygen in a carbon carrier-laccase system

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The influence of temperature, oxygen pressure and inhibitors of laccase on the dioxygen electroreduction reaction has been examined at different solution pH. On the basis of obtained data, a reaction mechanism including electron transfer from the enzyme active site to the oxygen molecule is suggested as the slow step.

Laccase; Cathode reduction; Oxygen; Electron transfer; Electronic energy level

1. INTRODUCTION

The ability of enzymes immobilized on electrode to accelerate electrode processes has been demonstrated for a number of reactions [1–3]. The reaction of oxygen electroreduction in the presence of immobilized laccase has been investigated most extensively. However, all the studies have been, as a rule, confined to a narrow range of potentials, and the mechanism of reaction was conjectured on the basis of Tafel's dependence within the potential range of 1.18–1.10 V.

Here we investigated the effects of electrolyte temperature and laccase inhibitors over a broad range of potentials and at different pH and oxygen pressure.

2. MATERIALS AND METHODS

Measurements were carried out with laccase adsorbed on carbon black. The latter was then pressed onto a porous disc 1 cm in diameter made of water-proofed carbon black. The catalyst was applied to the side immersed in electrolyte (1–2 mg per disc) which ensured equal availability of the surface [4]. The laccase was immobilized under steady-state conditions, from 1 ml of solution per 10 mg of carbon black in the course of 24 h at 4°C. The working and reference electrodes were thermostatted at the same temperature.

Specific activity of the enzyme was calculated taking into account a partial desorption of the enzyme (10–25%) after the temperature was raised from 15 to 45°C.

Laccase activity in phenoloxidase reaction was measured spectrophotometrically at 245 nm from the rate of quinone production, using citrate buffer as electrolyte.

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3. RESULTS AND DISCUSSION

The results are presented in figs 1–4. In the area of small polarizations, the dE/dpH value found from polarization curves of oxygen electroreduction at different pH (fig.1) is 0.060 V. In the region of maximum current the activity vs pH curve for the enzymatic electrode is bell-shaped, which is typical of the laccase phenoloxidase reaction rate dependence on pH.

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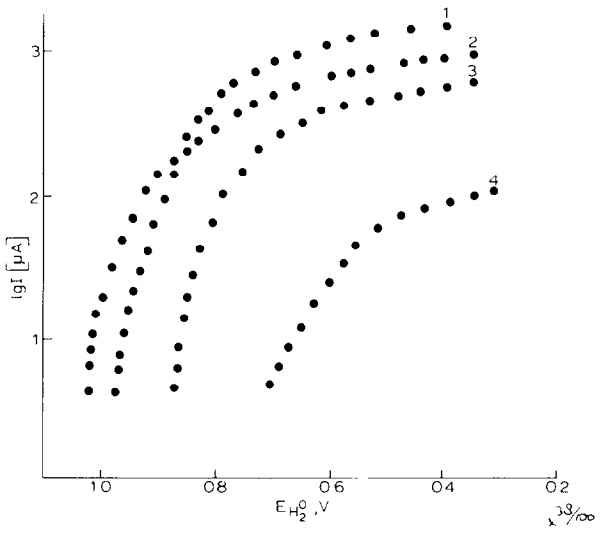

Fig.1. Polarization curves of oxygen electroreduction at an electrode with immobilized laccase in solutions with different pH: (1) −4.0; (2) −3.0; (3) −5.2; (4) −6.8.

The $dE/d\log P_{O_2}$ value for positive potentials greater than 1.10 V (relative to hydrogen electrode in the same solution) is 0.03 V, which is in agreement with the corresponding value of $dE/d\log I$ and points to a first order reaction for oxygen (fig.2, curve 1). At potentials more negative than 0.80 V the current vs partial oxygen pressure dependence (fig.2, curve 2) has the shape typical of the enzymatic reaction dependence rate vs substrate concentration (fig.2, curve 3).

The inhibitors studied (Cl^-, I^-, F^-) may, according to the inhibition mechanism, be divided into competitive with respect to the donor of electrons (Cl^-, I^-) and non-competitive (F^-) [5]. The effect of inhibitors in electrochemical reactions differs with polarization area. Chloride and iodide ions have almost no effect on the reaction rate in the region of maximum current (fig.3, curves 1,2) like in the enzymatic reaction. The effect of fluoride is of a more complex nature. At low polarizations the chloride and iodide ions compete with the electron donor (with electrode in this case) and inhibit the reaction more strongly with increasing concentration (fig.3, curves 1′,2′).

Based on the whole complex of the above data as well as on results from temperature studies (see below), it may be considered well founded that the rate of the process is limited by the electron

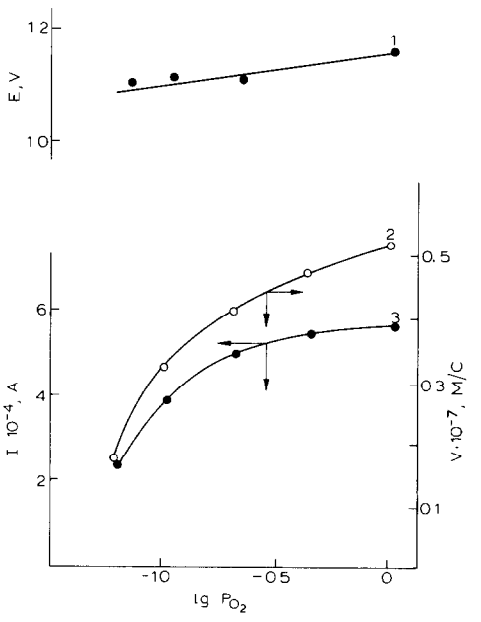

Fig.2. Potential and current of an electrode with immobilized laccase (1,3) and the rate of enzymatic oxidation of hydroquinone (2) as functions of oxygen partial pressure. Curve (1) for the region of low polarizations, curve (3) for the region of maximum current.

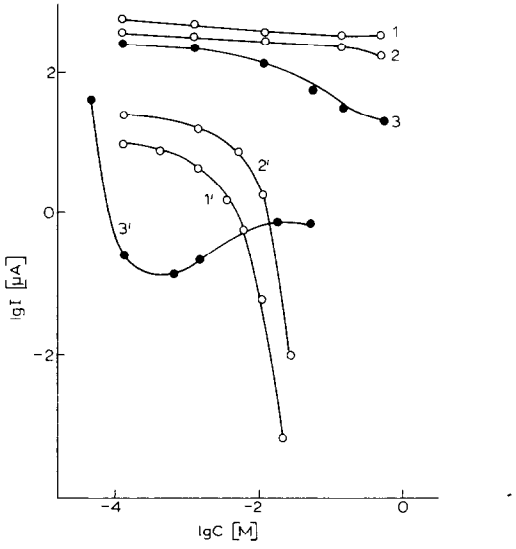

Fig.3. Effects of inhibitor concentration on the catalytic activity in the electroreduction of oxygen on an electrode with immobilized laccase. Curves (1–3) taken at E = 0.8 V; curves (1′–3′) at E = 1.15 V. (1,1′) In the presence of iodide, (2,2′) in the presence of chloride, (3,3′) in the presence of fluoride. Citrate buffer, pH 4.5.

transfer stage at low polarizations and by the stage typical of enzymatic catalysis in the region of maximum current. In the latter case the effects of various factors on the reaction rate (figs 1–3) are similar to those for the enzymatic reaction. At low polarizations the Tafel's dependence of reaction rate vs potential is observed (fig.1), indicating that electron transfer is the slow stage. The dependence of reaction rate on temperature follows the Arrhenius type over the entire range of potentials studied (fig.4). The activation energy at high polarizations (table 1) is close to that found for the phenoloxidase reaction of the immobilized enzyme (~10 kJ/mol); at low polarizations the activation energy is higher (~14 kJ/mol). Besides, a distinguishing feature of the process is the fact that the change of current with potential at low overvoltages is largely due to the change of the preexponential multiplier (table 1).

The observed dependences of the rates of elementary processes at low polarizations in this sytem may be accounted for by two possibilities: (i) a direct tunnel transfer of electron from the electrode to the enzyme's active center–oxygen complex, and (ii) transfer of electron from the active center to the oxygen molecule.

The obtained value of activation energy (table 1) as well as the evidence that tunnel transfer is not the slow stage for distances between the electrode and the catalytic site of less than 20 Å [6] suggest that the former possibility is less likely than the latter. The same conclusion is favored by the data on effects of inhibitor concentration on reaction rate (fig.3).

Table 1

Pre-exponential multiplier and activation energy values as functions of electrode potential

E (V)	1×10^{-3} A	A (kJ/mol)	K (A)[a]
1.15	0.4	14.4	0.4×10^{-3}
1.00	3.4	10.4	3.6×10^{-3}
0.90	6.9	10.3	7×10^{-3}
0.80	10.0	9.23	10×10^{-3}

[a] K values were calculated from the equation: $I = Ke^{-Ea/RT}$

The current values are given for 20°C, pH 4.5

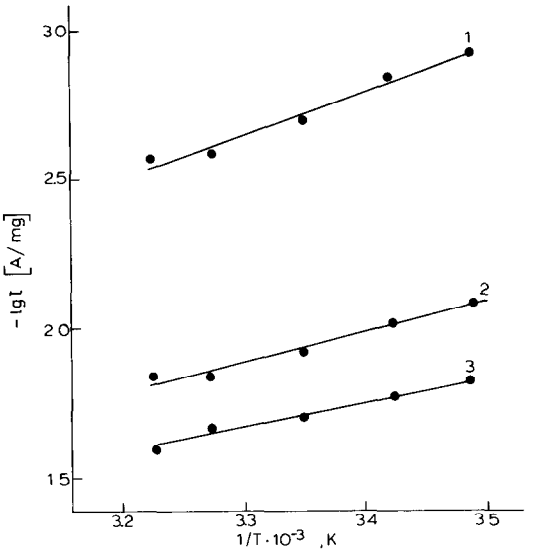

Fig.4. Arrhenius relationships of oxygen electroreduction on a laccase-immobilized electrode for potentials (V): (1) 1.15, (2) 1.0, (3) 0.9. Citrate buffer, pH 4.5.

According to the quantum theory of absorption, the chemisorption of atoms and molecules on metals or good conducting materials at an interphase with gas is accompanied by the conversion of local electron states into collectivized electron states of a system consisting of the metal and adsorbed particle [7]. This state differs from the chemical equilibrium between adsorbed molecule and electrode: here the average number of electrons at the adsorbed molecule is a function of the molecule's electronic energy level and does not depend, in an activational manner, upon temperature. It may be supposed that in our system the electron in the laccase active center does not react strongly with the solvent, and that no localized electron state is formed at the site [8].

Electron transfer processes involving delocalized adsorption states occur in a single elementary act which includes a direct exchange of electrons between the adsorbent-adsorbate system and the reagent in solution [9,10]. If we consider the electrode-immobilized laccase-oxygen system from this standpoint, the dependence of the preexponential multiplier upon electrode potential may be explained by the dependence of the electron level of the active center upon electrode potential, since the active center in this case is at a

considerable distance from the electrode surface (~10 Å), and the potentials at the electrode surface and at the active center are different.

The current of oxygen electroreduction at an electrode with immobilized laccase may be expressed as [9,10]:

$$I \sim \int d\epsilon \varrho_a(\epsilon) f(\epsilon) W(\epsilon) \sim \varrho_a(\epsilon^*) f(\epsilon^*) W(\epsilon^*) \quad (1)$$

where $f(\epsilon)$ is the Fermi electron distribution function; $W(\epsilon)$, probability of electron transfer from level ϵ at the active center to substrate molecule; ϵ^*, the value of energy which corresponds to a maximum contribution to the total current.

The electron state density associated with the active site may, in the simplest case, be expressed as follows [7]:

$$\varrho_a(\epsilon) = \frac{\gamma}{[\epsilon_a(E) - \epsilon]^2 + \gamma^2} \simeq \frac{\gamma}{[\epsilon_o - \epsilon - \mathcal{J}E]^2 + \gamma^2} \quad (2)$$

where $\epsilon_a(E) = \epsilon_o - \mathcal{J}E$ is electron energy in the active center; $\mathcal{J}E$, the potential drop between the surface and the active center; γ is proportional to the electronic matrix element which determines the electron exchange between the active center and metal.

The theoretical ideas discussed above make it possible to explain the obtained experimental data in the region of low overvoltages by assuming that a collectivized electronic state of the adsorbate-adsorbent type is formed in the immobilized laccase-carbon carrier system. In the region of maximum current, the limitation is due to a slow rate of the enzymatic reaction proper, rather than to attainment of the maximum rate of electron transfer from the active center of the enzyme to oxygen.

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