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Amperometric study of hydrogen peroxide biosensor with butadiene rubber as immobilization matrix

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ABSTRACT

A carbon paste electrode bound by butadiene rubber has been newly constructed and its electrochemical properties have been investigated to test the practicability of the enzyme electrode. The binder of carbon powder was butadiene rubber dissolved in toluene and ground cabbage tissue was embedded in the matrix as an enzyme source. The electrode, which showed a mechanical robustness after volatilization of solvent, displayed good catalytic power (detection limit = 2.5×10^{-5} M, S/N = 2) and electrochemically irreversible characteristics. Its symmetry factor and the exchange current density of the electrode used were 0.23 and 1.71×10^{-3} A cm^{-2}, respectively.

1. Introduction

It is essential to immobilize an enzyme on the surface of the biosensor so that it can completely preserve its catalytic power and specificity when one is trying to construct a biosensor and put it to practical use. This is due to an enzyme having the striking characteristic of accelerating reactions by a factor of at least a million, even in the case of lower temperature. A lot of methods have been introduced using mainly physical adsorption of an enzyme [1], a covalent bond with functional polymer [2], or polymer film for immobilizing an enzyme [3]. Also, the peculiar method, in which the source of enzyme is mixed with electrode material or doped into a sol–gel matrix [4], has been developed intensively up to the present. All the methods mentioned above should go through very tedious physical and chemical processes [5]. Those methodologies are lengthy processes, and therefore both costly and time-consuming for fabrication. In order to cut down on this ineffectiveness, electrodes using mineral oil for the binder of carbon powder had been designed and their electrochemical properties were studied in this lab [6]. Even now, this is very useful in the study of enzyme characteristics. However, since this cannot guarantee the mechanical robustness of the electrode, it is very far from being ready for practical use. In order to solve this problem with mineral oil, we have tried to find any binder which ensures the mechanical property of the enzyme electrode. We have confirmed that carbon paste exerts such mechanical stability once the solvent has completely escaped from the rubber solution after fabrication of electrode. These efforts led us to construct a lot of rubber electrodes using various kinds of rubbers such as ethylene propylene diene terpolymer [7], polybutadiene rubber [8], butyl rubber [9], and chloroprene rubber [10] as binders. Their usefulness was investigated and reported in journals several times. It is well known that butadiene rubber with sterical regularity is excellent in the aspect of flexibility and resistance to abrasion since it has no branch. Also, it is known that butadiene rubber has excellent dynamic properties and is good for its filling ability and compatibility [11]. Therefore, we expected that such characteristics might satisfy the mechanical robustness, which is a precondition for a practicable enzyme electrode, and made the electrode using BDR as binder. On the other hand, the peroxidase (Sigma, E.C.1.11.1.7) extracted from the horseradish was used as an enzyme source in the previous work. It is known that cabbage, being in the same family as cruciferae, contains lots of peroxidase. The ground cabbage tissue as an enzyme source was mixed directly with carbon paste in this system for economic reasons. In this study, the electrochemical properties of the enzyme electrode made in this way was inspected, and the result is reported in this paper.

2. Experiments

2.1. Materials

Butadiene rubber (abbr. BDR, Kumho petrochemical BR-01) was used as a binder and the source of enzymes was the ground root

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tissue of cabbage. Toluene and graphite powder were purchased from Sigma–Aldrich ($\geq$99.9%) and from Fluka ($\leq$0.1 mm) respectively. Hydrogen peroxide (Junsei, EP, 35%) for substrate (abbr. S), NaCl (Shinyo pure Chem., $\geq$99.5%) for electrolyte, and ferrocene (Sigma) for the mediator to increase and stabilize the signal, were used.

2.2. General procedures

After dissolving 0.09 g of ferrocene in 10 mL of $CHCl_3$, 0.91 g of the graphite powder was added and then dried. By mixing 1.0 g of the produced graphite powder with the solution of BDR solution (5.0%) at a 1:1 ratio (wt/wt), carbon paste was made. 1 g of this paste was completely mixed with 0.1 g of the ground cabbage root. The biosensor was constructed by packing this paste into a 6 mm i.d. and 1 mm depth polyethylene tube having ohmic contact. It was smoothed by friction on a spatula to make a flat working surface. The cyclic voltammograms were obtained in the states of both the unstirred and the stirred solution by the placement of the working electrode. The current response to the step-excitation was obtained as follows. When the decreasing tendency of the condenser current keeps horizontal after applying the step potential on the working electrode, substrate solution is added in 10 mL of 0.1 M NaCl solution. Then the current difference between before and after adding the substrate was considered to be the decomposition current of hydrogen peroxide. Ag/AgCl(BAS MF2052) and Pt electrode (BAS MW 1032) were used for reference and for auxiliary electrodes, respectively. The enzyme electrode was connected to a BAS Model EPSILON (Bioanalytical System, Inc., USA) to obtain cyclic voltammograms. The other amperometric measurements were performed with EG&G Model 362 potentiostat (Princeton Applied Research, USA). Its output was recorded on a Kipp & Zonen x-t strip chart recorder (BD111, Holland).

3. Results and discussion

Fig. 1 shows two different linear sweep voltammograms (LSV). A is one obtained in 0.1 M NaCl electrolytic solution and B, in 0.1 M NaCl solution which contains 1.0×10^{-3} M hydrogen peroxide. A consists of two kinds of reduction currents which show different increasing tendencies with electrode potential. One is increasing linearly below at -1.5 V and the other abruptly increasing above at -1.5 V. A glassy carbon electrode placed in 0.1 M NaCl electrolytic solution did not show any current in this range of potential [10]. The standard reduction potential of ferricinium ion is +0.400 V. It is probable that the tail of the reduction function of ferricinium would not have that much influence in the front part of the experimental range. During the process of electrolysis at -1.5 V or higher, one may observe air bubbles formed on the electrode surface by the naked eye. The standard reduction potential of water is -0.828 V. However, expecting the overpotential to be caused by a rubber component, the current which is increasing steeply above at -1.5 V may be considered to be the reduction current of water. BDR is a mixture composed of various chemical components even though their electrochemical behavior has not been elucidated here yet. Current occurring below at -1.5 V in Fig. 1 can be viewed as the reduction current of components of BDR. In Fig. 1, A is deformed to B by adding substrate into the electrolytic solution. Since this comes from the addition of H_2O_2 under the same conditions as A, it is clear that the reduction current produced by the decomposition of substrate created this deformation. The current difference between A and B is plotted with the applied electrode potential in Fig. 2. It is roughly symmetric. On the assumption that the reduction function of H_2O_2 has Gaussian distribution, the pictorial representation of the simulated result is a solid line. In the case of the complete reversible system, i_p^{rev} depends on the scan rate but E_p is independent of this. However, for the irreversible case, both i_p^{irrev} and E_p (peak potential) depend on $v^{1/2}$. In other words, E_p moves into the forward direction as v increases in the reduction reaction. The dependence of E_p on the scan rate in Fig. 3 tells us that the system is irreversible. The reaction between ferrous ion and hydrogen peroxide occurring in the organism is as follows:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^\bullet$$

and is one electron transfer reaction ($n = 1$) [12]. In the case of the irreversible system, kinetic parameters (i_0, α, etc.) of the electrode reaction may be obtained in the Tafel area. The relation between overpotential, E and $\ln\{(i_{l,c} - \mathrm{i})/i\}$ is linear and its slope and intercept are $\mathrm{RT}/\alpha\mathrm{nF}$ and $\mathrm{RT}/\alpha\mathrm{nF}(\ln_{i0}/i_{l,c})$ respectively.

Peak potential(E_p) representing the maximum current in Fig. 2 is -1.047 V. The plot of E vs. $\ln\{(i_{l,c} - i)/i\}$obtained by considering 0.349 mA at -1.047 V as $i_{l,c}$ is given in Fig. 4. The values of the slope and intercept worked out from Fig. 4 are 0.112 and -0.596, respectively. Making efficient use of those values, the calculated values of the symmetric factor (α) and exchange current density (i_0) are 0.23 and 1.71×10^{-3} A cm^{-2}, respectively. The value of $|E_p - E_{p/2}|$ is $47.7/\alpha n$ (mV) when the system is irreversible.

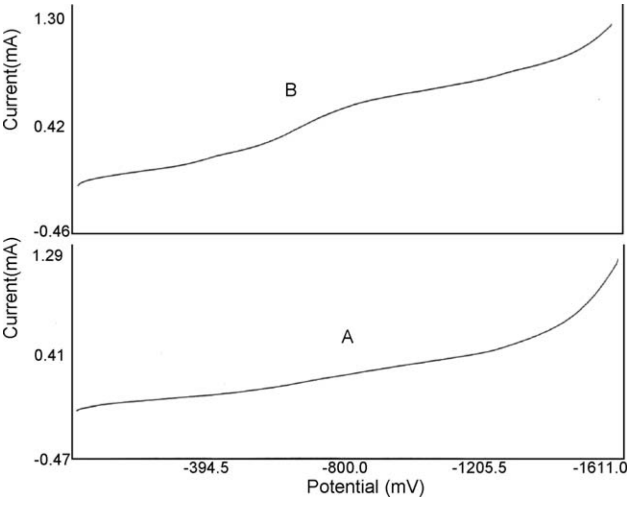

Fig. 1. The linear sweep voltammograms of the BDR-bound biosensor in the unstirred 0.1 M NaCl electrolytic solution in the absence (A) and in the presence (B) of 1.0×10^{-3} M H_2O_2. Initial potential: 0.0 V; scan rate: 0.1 V/s.

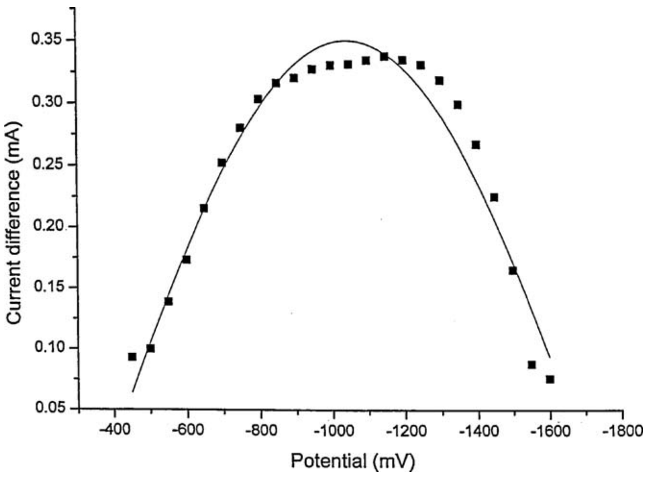

Fig. 2. Current difference between A and B in Fig. 1. Solid line is a Gaussian distribution (y = yo + (A/(w × sqrt(pi/2)))*exp(-2*((x-xc)/w)^2, yo: -0.884, xc: -1047, w: 1635, A: 2527) after performing the fit.