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단 신

Electrochemical kinetic analysis of the carbon paste enzyme electrode bound with butyl rubber

Keun-Bae Rhyu and Kil-Joong Yoon★

Division of Natural Sciences, Cheongju University, Cheongju 360-764, Korea

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부틸고무로 결합된 탄소반죽 효소전극의 전기화학 속도론적 고찰

유근배 · 윤길중★

청주대 자연과학부

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요 약: 톨루엔에 녹인 부틸고무 용액을 탄소가루의 결합재로 사용하였을 때, 탄소반죽은 용매 증발 후 기계적 경도를 보였다. 고무 용액의 이런 성질을 이용하여 닭의 간조직이 혼입된 새로운 과산화수소 정량 효소전극을 제조하였다. 그것이 전기화학적으로 정량적 행동을 보이는지 확인하기 위하여 그것의 전기화학적 파라메타 즉 이중층의 대칭인자, 교환전류밀도, 축전용량, Michaelis 상수 및 시간상수 등을 도출하였다. 실험 결과는 부틸고무 용액이 탄소가루의 결합재로 활용될 수 있음을 보여 주었다.

Abstract: When butyl rubber dissolved in toluene was used as a binder of carbon powder, carbon paste showed a mechanical hardness due to the fast volatility of the solvent just after the electrode fabrication. With a view of validating its quantitative electrochemical behaviors, its kinetic parameters, e.g. the symmetry factor, the exchange current density, the capacity of the double layer, the Michaelis constant, the time constant and other factors were investigated. Our experimental facts indicated that butyl rubber is available for a promising binder of carbon powder.

Key words: biosensor, redox enzyme, butyl rubber, hydrogen peroxide

1. Introduction

Carbon paste biosensors have obtained considerable popularity among chemists who devote intense study to the electrochemical properties of the redox enzyme. This methodology holds great attraction for biosensor chemists who hope to design the enzyme electrode at a moderate price in a short period of time. Its performance compares quite well with electrodes fabricated in other fields.[1-3]

★ Corresponding author
Phone : +82-(0)43-229-8540 Fax : +82-(0)43-229-8535
E-mail : kjyoon@cju.ac.kr

In this lab, enzyme electrodes using mineral oil as a binder of carbon powder were constructed and their electrochemical behaviors were studied.[4-6] To insure the practical use of the carbon paste electrode, a matter of the highest priority is that the electrode material should guarantee the mechanical solidity of the electrode.[7-8] But carbon paste electrodes using mineral oil as a binder are very far from being ready for practical use because such stability cannot be expected from mineral oil. In order to overcome this flaw, we have made efforts to find a binder that can ensure the mechanical properties of the enzyme electrode. As a result, we confirmed that carbon paste has such mechanical stability once the solvent has completely escaped from the rubber solution after the electrode's fabrication.

This revelation motivated us to construct a few rubber electrodes. Their usefulness has been reported several times.[9-10] For the enzyme electrode to be commonly applied in real life, the fundamental question to be ironed out is that of the qualitative and quantitative characteristic of the electrode.[6,12] It's because of its specificity and its immense catalytic power that the acceleration of reactions by a factor of at least a million should be experimented fully on the surface of the biosensor.

It is well known that the key features of butyl rubber are very low water absorption, good resistance to weathering, good tensile strength and abrasion resistance.[13] We expect that these properties of butyl rubber should satisfy the pre-requisite conditions for the practical use of the carbon paste electrode. An enzyme electrode for the determination of hydrogen peroxide was designed and its electrochemical behaviors were investigated. Here the context of this experiment is related along with its details.

2. Experimental

Butyl rubber was purchased from Exxon Chem. (Exxon Butyl 065, unsaturation avg. 0.7~1.0 mole %, U.S.A.). The toluene and carbon powder were products of Sigma-Aldrich (≥99.9%, U.S.A.) and Fluka (≤0.1 mm, Switzerland) respectively. Hydrogen peroxide (Junsei, EP, 35%) for substrate, NaCl (Shinyo pure Chem. ≥99.5%, Japan) for electrolyte, and ferrocene (Sigma) for mediator, were used and 0.1 M NaCl aqueous electrolytic solution was used in all measurements. After dissolving 0.09 g of ferrocene in 10 mL of $CHCl_3$, 0.91 g of carbon powder was added and then dried.

Carbon paste was made by mixing 1.0 g of the produced carbon powder with the butyl rubber solution (10% in toluene) at a 1:1 ratio (wt/wt). 0.065 g of chicken liver tissue triturated with homogenizer was embedded in 1.0 g of carbon paste immediately after its manufacturing. The enzyme electrode was constructed by packing this paste into a 6 mm inner diameter and 1 mm depth polyethylene tube with ohmic contact. The flat working surface of the electrode was obtained by friction on a spatula.

The working electrode placed in the unstirred solution was connected to a BAS Model EPSILON (Bioanalytical System, Inc., U.S.A.) to obtain the LSV (linear sweep voltammogram). The other amperometric measurements were performed on EG & G Model 362 potentiostat (Prinston Applied Research, U.S.A.) and its output was recorded on a Kipp & Zonen x-t strip chart recorder (BD111, Holland). Pt (BAS MW 1032) and Ag/AgCl (BAS MF 2052) were the counter and the reference, respectively. Amperometric signals were measured as follows. When the decreasing tendency of the residual current stayed level after the step-potential excitation, substrate was added to 10 mL 0.1 M NaCl solution. Then the current difference between before and after adding the substrate was considered to be the reduction current of the substrate. Origin 7 was used for all the calculations.

3. Results and Discussion

An enzyme electrode designed to examine the electrochemical characteristics of the mediated reduction current has four structural materials. In order to observe the behaviors of each component and to set up the experimental potential range without electrochemical disturbance, four different electrodes were constructed and their profiles are shown in *Table* 1.

Table 1. Electrodes for evaluating the electrochemical properties of the structural materials

Electrode	Structural elements, (g)
A	cp(0.5) + bt(0.5)
B	cp(0.5) + bt(0.5) + tis(0.065)
C	cp(0.5) + bt(0.5) + fer(0.09)
D	cp(0.5) + bt(0.5) + tis(0.065) + fer(0.09)

cp: carbon powder; bt: butyl rubber solution (10% in toluene); tis: chicken liver tissue; fer: ferrocene.

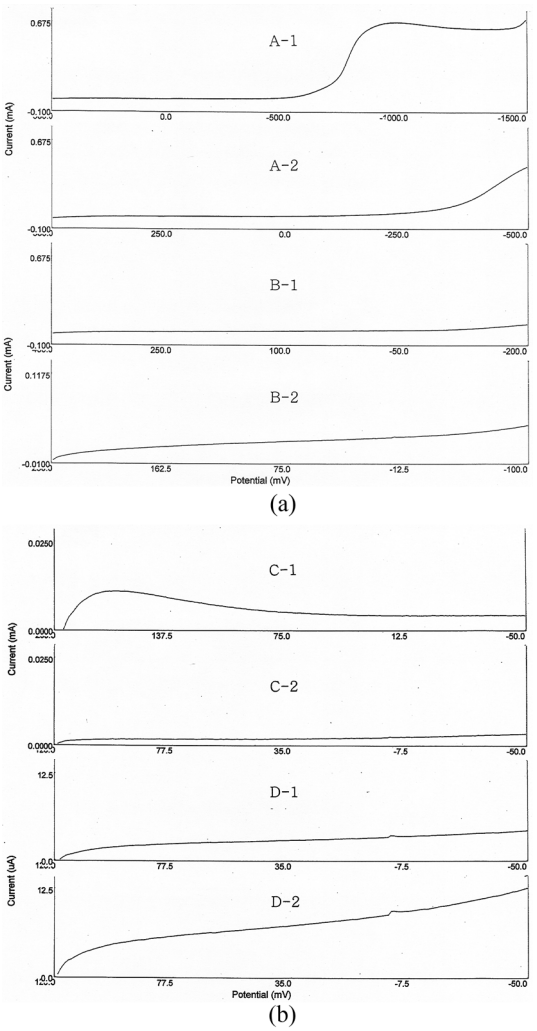

Fig. 1. Linear sweep voltammograms for elucidating the electrochemical behaviors of the structural elements in biosensor: (a) in the absence of substrate and (b) in the presence of 2.0×10^{-2} M H_2O_2. Scan rate: 50 mV/s.

Fig. 1 shows all procedural matters connected with discerning their electrochemical activities.

In *Fig*. 1, A-1 shows the LSV of the electrode A in the absence of substrate. It depicts a very large cathodic current at above -500 mV. Electrode A has two elements, butyl rubber and carbon powder. Butyl rubber is a cationic polymer of which the active species is a carbonium during polymer chain growth.[13] Because the ordinary glassy carbon electrode did not show any reductive wave in the same condition as above,[14] the current in the said potential range can be viewed as the reduction current of unidentified components in butyl rubber.

After the potential range was narrowed down to -500 mV, A-2 was obtained in the presence of substrate (0.02 M.H_2O_2). It can be seen that the reduction current appears at above -250 mV. The axis of ordinates in A-1 and A-2 is the same current level and this was not entirely seen in the former. The reduction of the substrate is achieved in two ways in this system. One is to directly reduce the substrate using electrons springing up on the electrode surface at high electrode potential and the other is by mediation at low electrode potential. The reduction current appearing in A-2 belongs to the former and is treated as a product of side reactions in this work. So, the potential range at above -200 mV should be eliminated from consideration because our attention is focused on the latter.

B-1 was obtained in the potential without influence from the binder or direct reduction in order to examine the electrochemical behavior of the tissue. This shows an immaterial increase of reduction current at above -100 mV though there is no substrate in the electrolytic solution. This phenomenon indicates that some of the unidentified components contained in the tissue are electrochemically active in the region.

B-2 describes an LSV obtained in +250 ~ -100 mV when the substrate is added. It is magnified 6 times on the y-scale. The anodic and cathodic current flow occurs at the positive and negative end of the electrode potential, respectively. It is probable that the cathodic current on the right is the tail of the reduction function in B-1, but there is no theoretical justification to explain the appearance of the anodic current on the left yet. It is common knowledge that

the reversibility of ferrocene is excellent.

Electrode C is fabricated to examine the possible effect of ferrocene on the mediated current. Although C-1 doesn't contain substrate, it shows strong reduction peak at below +120 mV. Our experience tells us that this peak is a result of the reduction of ferrocene. A pure mediated current should be considered within the potential range without interference from ferrocene. C-2 is obtained in an electrolytic solution with the substrate, which runs from +120 to -50 mV. In spite of the presence of the substrate, it is very horizontal and doesn't show any signs of side reactions. So to say, this is the potential range free from electrochemical disturbance in structural elements. D-1 was acquired in the absence of the substrate. Here the graduation of the current was doubled. It shows a small residual current increasing with the electrode potential. Considering that the condenser current is linear with the potential, it is reasonable to regard this as the current charging the double layer. The extent of noise like this is unavoidable in electrochemistry. No sooner was the substrate added than D-2 shows an increasing current throughout the length and breadth of electrode potential. This change was caused by the addition of substrate under the condition that all effects of electrode materials were eliminated. Because the residual current is common to both LSV's, the current difference between D-1 and D-2 can be regarded as the mediated reduction current of substrate.

The current difference is illustrated with the electrode potential in *Fig*. 2. The electrochemical reaction is diffusion-controlled and the current-voltage profile is commonly sigmoidal.[2,15] Because the electrode potential can not be expanded beyond -50 mV, the Boltzmann fitting was carried out locally and its resulting functional relation is as follows:

$$i = 7.00+(191.83-7.00)/\{1+\exp(E+213.83)/81.51\}$$

where i is the current density ($\mu A/cm^2$) and E is the applied potential (mV). The limiting value of i is 191.83 $\mu A/cm^2$ on the right, which is regarded as the limiting current, $i_{l,c}$.

The relation between over potential and ln $(i_{l,c}-i)/i$ is linear (*Fig*. 3) in the irreversible system and has the slope, RT/ánF and intercept, RT/αnFln($i_o/i_{l,c}$). The reaction between ferrous ion and hydrogen peroxide occurring in the organism is one electron transfer reaction (n=1).[16] Being analyzed with two Tafel constants in *Fig*. 3, symmetry factor, α and exchange current density, i_o, are 0.19 and 22.6 $\mu A/cm^2$, respectively. The symmetry factor is smaller than the others.[10,11] It says that the height of the activation energy barrier formed in the double layer is more effectively adjustable in the butyl rubber electrode than in the others.

A calibration curve obtained using successive hydrodynamic amperometry is given in the inset of *Fig*. 4. It is usual with the enzyme electrode that

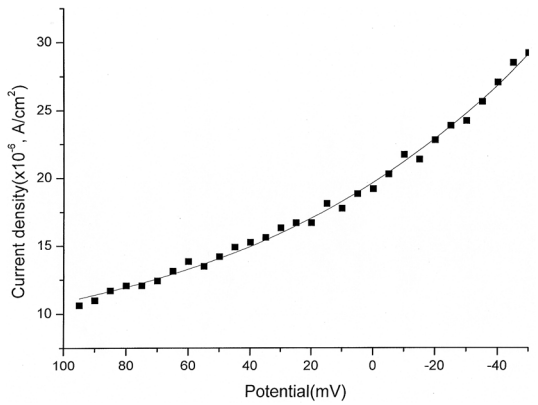

Fig. 2. Potential dependence of the mediated current. Every point was calculated using the values read out by eye measure on the zoomed LSV's.

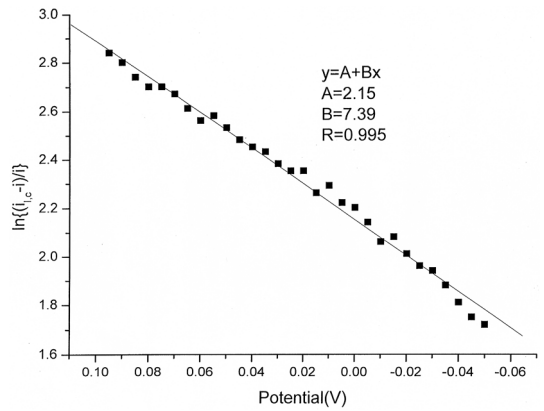

Fig. 3. Linear plot of ln{($i_{l,c}$-i)/i} vs. E for determining the Tafel constants.

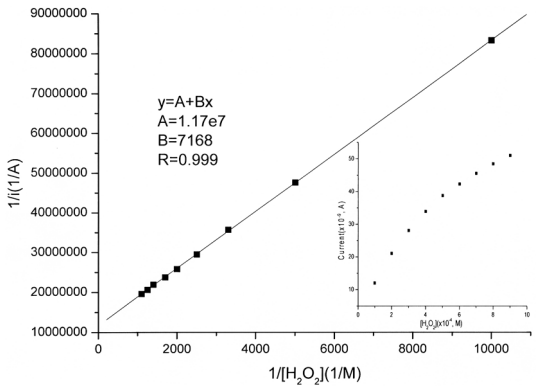

Fig. 4. Enzyme kinetic analysis of the mediated current. Inset: plot of the cumulative amperometric response electrode on successive additions of substrate at -50 mV(vs. Ag/AgCl).

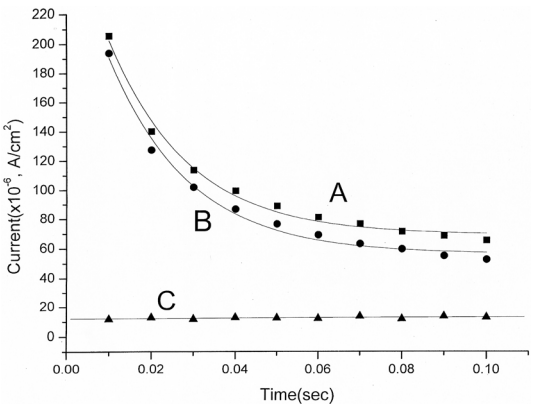

Fig. 5. Current transient obtained from the solution with(A) and without(B) the substrate (0.02 M H_2O_2) at -50 mV. C: current difference between A and B.

calibration deviates easily from their linearity even at low concentration.[17] Several explanations are possible for this phenomenon. First, the active sites on the electrode surface are limited in number. It is probable that the enzyme can catalyze at any one of the steps when the dissociation of hydrogen peroxide goes through multi-step reaction. Besides, various kinds of isoenzyme[1] can take part in hydrogen peroxide dissociation at various rates. If the double reciprocal plot of signal and concentration is linear, then it recognizes that the reaction is by catalysis of the enzyme. *Fig.* 4 is a Lineweaver-Burk plot obtained by taking the reciprocals of the accumulated currents and the accumulated concentrations of the calibration. It shows good linearity with a correlation of R^2=0.999. This indicates that the dissociation of hydrogen peroxide is being catalyzed by the peroxidase in chicken liver. The maximum current (i_{max}) and Machaelis constant (K_M) calculated from the plot are $8{,}55\times10^{-6}$ Acm^{-2} and 6.13×10^{-4} M respectively.

A cell in the IPE system is represented by the electrical circuit of a resistor (R_s) representing the solution resistance and capacitor C_d representing the double layer in series. The decay pattern of the condenser current is 1-st order exponential. *Fig.* 5 shows two current transients (i *vs*, t) obtained in the electrolytic solution with (A) and without (B) added substrate (0.02 M H_2O_2). The relationship between current and time simulated since 0.01 sec after potential step is

$$i = 227.75\exp(-t/0.01879) + 68.39$$

Current value, $i_{max,B}$, at t=0, which couldn't be achieved experimentally, is determined by the extrapolation of the tendency to t=0. $i_{max,B}$ and the solution resistance obtained from this relation were 296.14 μA/cm^2 at t=0, and 169 Ω respectively.

Besides, the time constant of the current transient, τ_B, is computed to be 0.032 seconds. This comes from applying the upper relation and the capacitance of the double layer, which is obtainable using the equation, $\tau_B=R_sC_d$, is 1.89×10^{-4} F. $i_{max,A}$ and τ_A derived from A in similar fashion are 284.39 μA/cm^2 and 0.029 sec. We can understand the relations, $i_{max,B} > i_{max,A}$. and $\tau_B > \tau_A$. This means that the migration of electrolytes is retarded by the neutral substrate in the beginning of potential step. Here the difference between A and B is constant with time after the time constant. It indicates the system is diffusion-controlled.

4. Conclusion

The enzyme electrode showed an excellent solidity and its average prospective life was semi-permanent. The results demonstrated that peroxidase in carbon paste bound with butyl rubber functions qualitatively and quantitatively. Those indicate that butyl rubber is a recommendable binder for the development of the

practicable carbon paste electrode. However, the activation energy which is required to deform the three-dimensional structure of the enzyme is 16~60 kJ/mol and surroundings like this are probable in room condition. If further study for the safekeeping of the enzyme electrode were carried out, this electrode could be a valuable analytical appliance.

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