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Glucose Sensitivity of Thiol-modified Gold Electrodes Having Immobilized Glucose Oxidase and 2-Aminoethylferrocene

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A novel amperometric glucose sensor was fabricated in which glucose oxidase and 2-aminoethylferrocene as an electron mediator were chemically bound to self-assembled 4-aminothiophenol monolayer on gold electrode with use of glutaraldehyde as a cross linking agent. The glucose sensor prepared under optimal conditions showed sensitivities more than 30 $\mu A\ cm^{-2}$ for 20 mM of glucose.

Membrane free amperometric enzyme sensors with covalently bound enzyme to carbon electrode substrates[1,2] is of interests in the point that they have in principle high amperometric sensitivities free from any diffusion problem of substrates or products through the enzyme membrane. Prototypes of this kind of glucose sensors reported so far utilize covalently bound glucose oxidase (**GOx**) alone, and an appropriate mediator has to be present in the test solutions for the prepared sensor to show amperometric sensitivity. We have studied amperometric glucose sensors based on electrically conducting polypyrrole films in which both **GOx** and an electron mediator are electrostatically fixed.[3,4] As the electron mediators, we have used hydroquinone sulfonate and ferrocenecarboxylate. The immobilization of the electron mediator and **GOx** in polypyrrole films allowed amperometric detection of glucose without use of any electron mediators in the test solutions. However, the amperometric sensitivities are mostly determined by the rate of diffusion of glucose in the conducting polypyrrole films. To solve this problem, we have attempted to fabricate a new type amperometric glucose sensor using self-assembled 4-aminothiophenol (**AT**) films to which both GOx and an electron mediator are covalently bound. Self-assembled monolayer films are one of recent topics in interface chemistry including electrochemistry.[5]

It has been reported that chemical bindings of enzymes such as **GOx**[2] and diaphorase[6] to self-assembled films can be made using glutaraldehyde as a cross-linking agent. In these cases, amino residues of enzymes are utilized for binding the enzymes with glutaraldehyde. To bind an electron mediator to glucose oxidase, it is desired for the electron mediator to have amino groups. In the preset study, 2-aminoethylferrocene (**AF**) was chosen for this purpose. The **AF** was found to have a higher ability for electron mediation to **GOx** than hydroquinone sulfonate and ferrocene carboxylate which were used in the previous studies.[3,4] If the chemical binding ideally occurs, we can postulate the structure of the prepared electrode as illustrated in Fig. 1. As will be shown below, however, multilayers of **GOx** and **AF** were formed on the electrode surface by the immobilization method adopted in this study.

A gold plate was used as an electrode substrate after polishing with alumina. The electrode substrate was

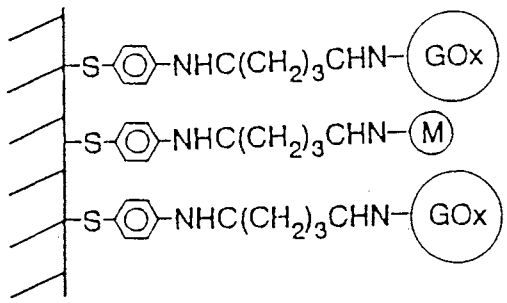

Fig. 1. Molecular structure of GOx/AF/AT/Au electrode. GOx: glucose oxidase. M: electron mediator.

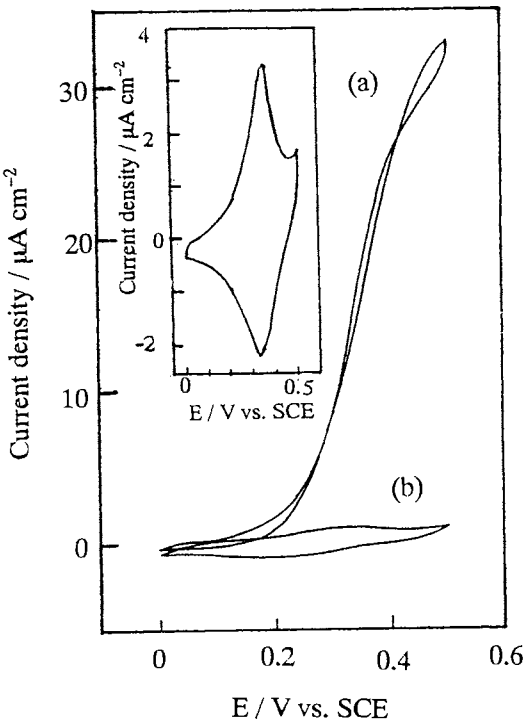

Fig. 2. Cyclic voltammograms of GOx/AF/AT/Au electrode in 0.1 M phosphate buffer (pH 7) in the presence (a) and absence (b) of 38 mM glucose. dE/dt = 10 mV s^{-1}.

immersed overnight in aqueous solution containing 1 mM (1 M = 1 mol dm^{-3}) **AT** to prepare a self-assembled **AT** monolayer on the **Au** electrode. The resulting electrode is denoted here as **AT/Au** electrode. After washing with twice distilled water, the **AT/Au** electrode was again incubated at 30°C for 2 h in aqueous solution containing 1 g dm^{-3} **GOx**, 5 mM **AF**, and 7% glutaraldehyde. The electrode prepared in this way is denoted here as **GOx/AF/AT/Au** electrode. It was washed and immersed in 0.1 M phosphate buffer solution (pH 7) for 1 h to remove physically bound **GOx** and **AT** on the electrode.

The **GOx/AF/AT/Au** electrode showed amperometric sensitivities to glucose, as recognized from current potential curves shown in Fig. 2. By adding glucose to the phosphate buffer solution, anodic currents were greatly enhanced, indicating that the reactions given by Eqs. (1)-(3) successively occurred,

$$\mathbf{GOx}_{ox} + \text{glucose} \rightarrow \mathbf{GOx}_{red} + \text{gluconolactone} \quad (1)$$

$$\mathbf{GOx}_{red} + \mathbf{AF}_{ox} \rightarrow \mathbf{GOx}_{ox} + \mathbf{AF}_{red} \quad (2)$$

$$\mathbf{AF}_{ox} \rightarrow \mathbf{AF}_{ox} + e^- \quad (3)$$

where the suffix ox and red denote oxidized and reduced form, respectively. The inset of Fig. 2 shows a cyclic voltammogram of the **GOx/AF/AT/Au** electrode taken in a glucose-free phosphate buffer solution. The redox potential of the immobilized **AF** is evaluated to be 348 mV vs. SCE, which is about 150 mV more positive than that obtained in dissolved state.[7] Since the potential separation between the anodic current peak and cathodic one is as small as 15 mV, the **GOx/AF/AT/Au** electrode is said to behave like a thin layer film-coated electrodes showing high charge transport diffusion in the film layer.[7]

The prepared electrode showed apparent enzyme activities of 200 mU cm^{-2} as determined by the indamine dye assay described previously.[3] In order to estimate the amount of **GOx** immobilized, the electrode was immersed in 8 mol dm^{-3} urea solution to release FAD from **GOx**, followed by measuring the fluorescence

intensity of the resulting solution at 525 nm with the excitation wavelength at 370 nm.[3] It was found by this experiment that 4.15 x 10^{-11} mol cm^{-2} of **GOx** was immobilized on the electrode surface. Since a **GOx** molecule has diameter of 180 Å,[9] the highest density of its monolayer was calculated to be 6.53 x 10^{-13} mol cm^{-2}. By comparing this value with the estimated amount of **GOx** of the **GOx/AF/AT/Au** electrode, it was speculated that **GOx** and **AF** were immobilized on the electrode with 64 layers at least. Such multilayers can be formed if glutaraldehyde make bridges between **GOx** enzymes each of which has many residual amino groups. The bindings of **AF** molecules to **GOx** can also occur in the same way using the amino group of **AF**. The amount of **AF** immobilized to the electrode substrate was estimated based on the charges involved in the redox waves of the **GOx/AF/AT/Au** electrode given in the inset of Fig. 1. The obtained redox charge was 0.0486 mC cm^{-2}, indicating that the amount of **AF** immobilized was 5.04 x 10^{-10} mol cm^{-2} which was about 10 times as large as that of **GOx** immobilized.

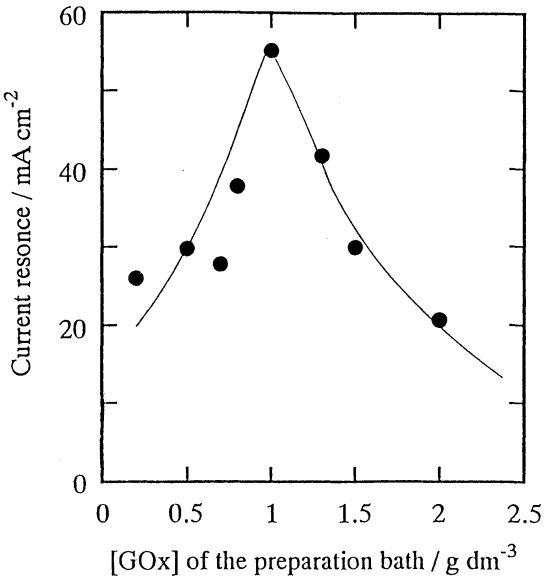

Fig. 4. Effect of the concentration of GOx in the preparation bath of GOx/AF/AT/Au electrode on the current response to 30 mM glucose. The concentration of AF in the preparation bath was fixed to 5 mM. E = 0.35 V vs. SCE.

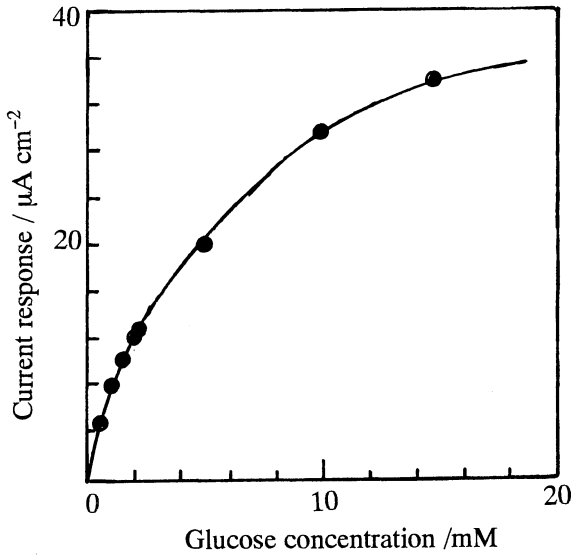

Fig. 3. Calibration curve of GOx/AF/AT/Au electrode for amperometric detection of glucose at 0.35 V vs. SCE.

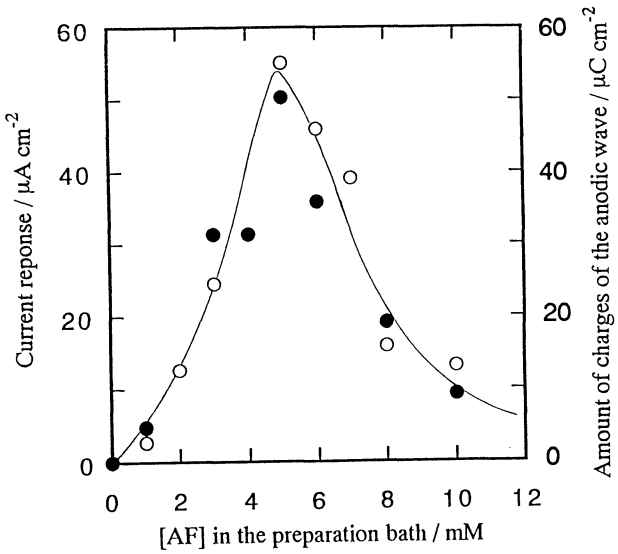

Fig. 5. Effect of the concentration of AF in the preparation bath of GOx/AF/AT/Au electrodes on the current response to 30 mM glucose (O) and the amount of charge of the anodic wave of cyclic voltammograms taken at the sweep rate of 10 mV s^{-1} (●). The concentration of GOx in the preparation bath was fixed to 1 g dm^{-3}.

Figure 3 shows a calibration curve of the **GOx/AF/AT/Au** electrode for amperometric detection of glucose. More than 30 μA cm^{-2} of anodic currents were obtained for 20 mM glucose, the sensitivity of which was much greater than that obtained at polypyrrole based glucose sensors investigated previously. The Eadie–Hofstee plots[10] of the results given in Fig. 3 allowed the determination of the apparent Michaelis Menten constant of 3.1 mM. The current response as shown in this figure was not observed when either fructose or galactose was added to the phosphate buffer solution, indicating that **GOx** retained its reaction selectivities after binding to the **AT/Au** electrode.

The above results were obtained for the electrode prepared with use of 5 mM **AF** and 1 g dm^{-3} **GOx** in the preparation bath of the electrode. If the concentration of **GOx** in the bath was changed but that of **AF** was unchanged, anodic currents due to oxidation of glucose of the resulting **GOx/AF/AT/Au** electrodes were changed, as shown in Fig. 4. Similarly, if the concentration of **AF** of the preparation bath was changed with fixing the concentration of **GOx** at 1 g dm^{-3}, the amount of charges involved in oxidation of the immobilized **AF**, determined by cyclic voltammetry, was changed as shown in Fig. 5 by filled-in circles. Simultaneously, the anodic currents due to oxidation of 30 mM glucose was changed as given by open circles of the same figure. The results given in this figure suggest that the glucose sensitivity is mainly determined by the redox activity of **AF** in the **GOx/AF/AT/Au** electrodes. The decrease in the amount of redox charges and the glucose sensitivity beyond 5 mM of **AF** in the preparation bath may suggest that with increasing the concentration of **AF** beyond this threshold value, the binding between **AF** and glutaraldehyde took place preferentially, causing a decrease in the amount of glutaraldehyde available for making bridges between **GOx** enzymes to form multilayers. Detailed analysis of the compositions of the **GOx/AF/AT/Au** prepared in the present study are under way.

References

1) R. M. Ianniello and A. M. Yacynych, *Anal. Chem.*, **53**, 2090 (1981); G. Johnson and L. Gorton, *Biosensors*, **1**, 355 (1985).
2) T. Tatsuma and T. Watanabe, *Anal. Chem.*, **64**, 625 (1992).
3) C. Iwakura, Y. Kajiya, and H. Yoneyama, *J. Chem. Soc., Chem. Commun.*, **1988**, 1019; Y. Kajiya, H. Matsumoto, and H. Yoneyama, *J. Electroanal. Chem.*, **319**, 185 (1991); Y. Kajiya and H. Yoneyama, *J. Electroanal. Chem.*, **314**, 85 (1992).
4) Y. Kajiya, H. Sugai, C. Iwakura, and H. Yoneyama, *Anal. Chem.*, **63**, 49 (1991).
5) See, for example, M. D. Porter, T. B. Bright, D. L. Allara, and C. E. D. Chidsey, *J. Am. Chem. Soc.*, **109**, 3359 (1987); C. D. Bain and G. M. Whitesides, *ibid.*, **110**, 3665 (1988); C. A. Widrig, C. A. Alves, and M. D. Porter, *ibid.*, **113**, 2805 (1991); H. O. Finklea, S. Avery, M. Lynch, and T. Furtsch, *Langmuir*, **3**, 409 (1987); C. Miller, P. Cnendet, and M. Grätzel, *J. Phys. Chem.*, **95**, 877 (1991).
6) T. Sawaguchi, T. Matsue, and I. Uchida, *Bioelectrochem. Bioenerg.*, **29**, 127 (1992).
7) M. J. Green and H. A. O. Hill, *J. Chem. Soc., Faraday Trans. 1*, **82**, 1237 (1986).
8) R. W. Murray, "Electroanalytical Chemistry," ed by A. J. Bard, Dekker, New York (1984), Vol. 13, p. 191.
9) R. Messing, *Biotechnol. Bioeneg.*, **16**, 897 (1974).
10) R. A. Kamin and G. S. Wilson, *Anal. Chem.*, **52**, 1198 (1980).

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