CHEMISTRY LETTERS, pp. 1183-1186, 1988.

Glucose Oxidase-Modified SnO_2 Electrode as Electrochemical Glucose Sensor

Tadashi WATANABE,* Yusuke OKAWA, Hirohiko TSUZUKI, Shoichiro YOSHIDA, and Yoshimasa NIHEI

Institute of Industrial Science, The University of Tokyo, Roppongi, Minato-ku, Tokyo 106

Glucose oxidase was chemically immobilized on an SnO_2 electrode via 1) 2,4,6-trichloro-1,3,5-triazine, 2) 3-aminopropyltriethoxysilane and glutaraldehyde, or 3) crosslinkage of enzyme molecules by glutaraldehyde. These enzyme-immobilized electrodes were compared regarding the performance as amperometric glucose sensors.

Conventional bioelectrochemical sensors based on amperometric detection are usually fabricated with metal substrate electrodes such as Pt[1—3] and Au.[4] The surface of these metals, however, is not sufficiently stable.[5] Thus, for biosensors with such metal electrodes, pretreatment and measuring procedure itself often affect seriously their electrochemical behavior and hence the sensor performance, thus such sensors tend to show relatively low reproducibility on repeated use. The choice of an electrode material is of much importance in biosensor construction.

SnO_2 is one of the materials featuring high electrical conductivity, chemical stability, and the ease in chemically modifying the surface with functional compounds. In view of this, many works have ever been carried out on the chemical modification of SnO_2 electrodes.[6—9] To date, however, application of these advantageous characteristics of SnO_2 electrodes to the fabrication of biosensors has never been attempted. The present work is the first demonstration of a biosensor with an SnO_2 electrode with chemically immobilized enzyme molecules.

The relationship between the surface state of chemically immobilized enzyme molecules and sensor performance has scarcely been studied. In view of this, we have also tried in the present work to clarify the factors dominating the sensor performance from the comparison of results obtained for sensors prepared in different manners.

SnO_2-coated glass plates were used as sensor substrates. Glucose oxidase (GOD, EC 1.1.3.4, from *Aspergillus niger*) was covalently attached to the SnO_2 surface by either of the following two methods: 1) An SnO_2 plate was treated with saturated toluene solution of 2,4,6-trichloro-1,3,5-triazine (cyanuric chloride) for 2 h at room temperature, rinsed with toluene, and then immersed in a phosphate-buffered GOD solution (30, 140, 570, or 1100 $U \cdot cm^{-3}$,[10] pH 7) for 30 min at room temperature [cyanuric chloride method];[11] 2) An SnO_2 plate was treated successively with a 10% aqueous solution of 3-aminopropyltriethoxysilane for 2 h at 50 °C, a 2.5% glutaraldehyde aqueous solution for 1 h at room temperature, and then a GOD solution (200 $U \cdot cm^{-3}$) for 1 h at room temperature [silane coupler method].[12] A

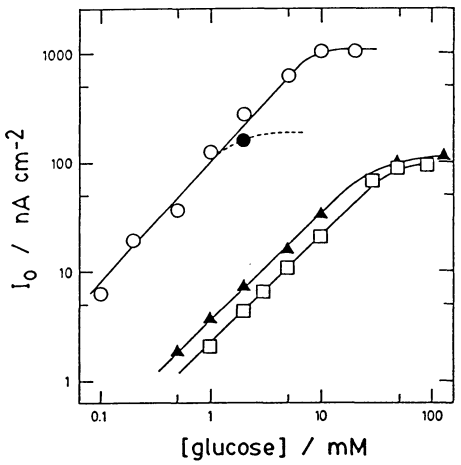

Fig. 1. Output current (I_o) *vs.* glucose concentration profile for three types of sensors: □, cyanuric chloride method sensor; ▲, silane coupler method sensor; ○, crosslinked membrane sensor. For the crosslinked membrane sensor, oxygen was periodically bubbled to the test solution at glucose concentrations above 1 mM (●, without further O_2-supply).
Electrode potential = +0.90 V *vs.* Ag/AgCl.

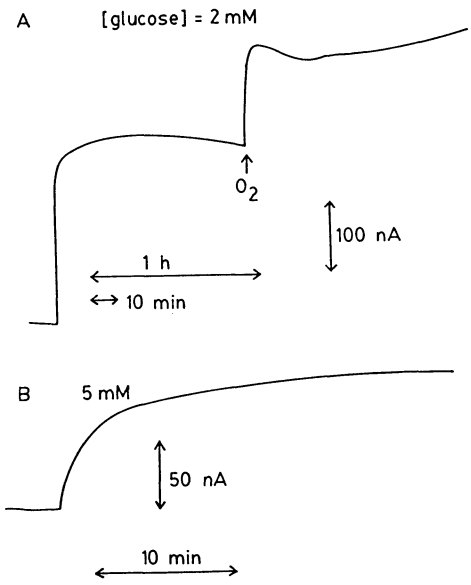

Fig. 2. Time response of the sensors. A, crosslinked membrane sensor; B, silane coupler method sensor.

crosslinked GOD membrane electrode was prepared by casting a GOD/glutaraldehyde mixed solution onto an SnO_2 plate pretreated with 3-aminopropyltriethoxysilane. These enzyme-immobilized electrodes were stored in a phosphate buffer at 4°C before use.

The sensor signal was obtained as electrochemical oxidation current of enzymatically formed hydrogen peroxide in a pH 6.4 phosphate buffer with a conventional three-electrode system at room temperature. Prior to measurement, each test solution was flushed with gaseous oxygen.

Figure 1 shows typical responses of the three types of the GOD-immobilized sensors for the glucose concentration in test solution. These responses are stable and reproducible without any electrochemical surface treatment such as cleaning or activation.[1,4] In measurement of the electrochemical oxidation current of H_2O_2 with conventional metal electrodes, the current arising from surface oxide formation frequently overlaps the sensor output. This surface oxidation current depends on many factors such as the initial state of the metal surface, time, and dissolved substances in the solution. The SnO_2-based sensors, in contrast, shows neatly the H_2O_2 oxidation current with a small back ground current from solvent oxidation. This observation demonstrates the advantage of the use of a chemically stable substrate as electrode.

The crosslinked GOD membrane sensor exhibits the highest sensitivity, but the linear response range is limited to glucose concentrations below about 1 mM (1 M = 1 mol·dm^{-3}) without further oxygen supply. Periodical oxygen bubbling is required to obtain a linear response in a concentration range above 1 mM. Obviously the

response saturation is caused by oxygen depletion at the membrane/solution interface. Restoration of the sensor signal to a normal level by oxygen bubbling, is depicted in Fig. 2A. In contrast, the sensors with covalently attached GOD exhibit a much wider dynamic range, that is, a linear response up to a glucose concentration of 30 - 50 mM, without further oxygen supply.

The sensitivity of the cyanuric chloride method sensor decayed to about half the original level within the initial 24 h after preparation, but remained nearly constant for at least 3 months thereafter. The initial decay may reflect some conformational change in the surface-attached GOD molecules, but elucidation of its mechanism is beyond the scope of the present communication.

In order to characterize enzyme sensors basically, it is necessary to clarify the factors affecting the sensor response. Here, we have attempted such characterization using GOD-modified SnO_2 electrodes.

The sensitivity (S) of a glucose sensor, defined as the gradient of a linear portion of the output current density vs. glucose concentration profile, should be proportional to a product of the GOD specific activity (α), surface density (σ), and a factor ϕ, being the fraction of enzyme molecules which can supply the electroactive species (H_2O_2) to the electrode surface over the total immobilized enzyme molecules.

$$S = k\alpha\sigma\phi \qquad (1)$$

Here k is a proportionality constant. As the chemical modification reaction is considered to be a kind of chemisorption process, σ could be related to the GOD concentration (C_E) in the surface modification bath with a Langmuir-type equation;

$$\sigma = \sigma_{max} C_E (C_{1/2} + C_E)^{-1} \qquad (2)$$

where σ_{max} is the maximum enzyme density and $C_{1/2}$ is the Langmuir concentration. Combining Eqs. 1 and 2, the following formula is obtained;

$$C_E/S = S_{max}^{-1}(C_E + C_{1/2}) \qquad (3)$$

where $S_{max} = k\alpha\phi\sigma_{max}$. For a series of sensors prepared by the same modification procedure varying C_E, α and ϕ, and hence S_{max} should be constant, then the C_E/S *vs.* C_E relationship will give a linear plot. S_{max} represents the maximum sensitivity of the sensor prepared by this method. Fig. 3 gives the experimentally found S *vs.* C_E and C_E/S *vs.* C_E relationships for a series of sensors prepared by the cyanuric chloride method. The excellent linear relationship obtained for the C_E/S *vs.* C_E plot verifies the above speculation. We can evaluate from this plot directly the maximum sensitivity (S_{max}) and the quantitative index of C_E for sufficient sensitivity ($C_{1/2}$).

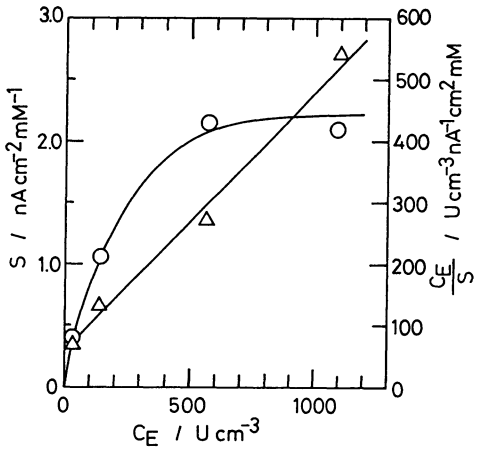

Fig. 3. S *vs.* C_E (○) and C_E/S *vs.* C_E (△) profiles for the cyanuric chloride method sensor.

Chemical analysis[13] showed that the surface density of covalently immobilized GOD molecules is on the order of 2×10^{13} molecules·cm^{-2} in either the cyanuric chloride or the silane coupler method. This value indicates that GOD molecules are attached in the state of a closely packed monolayer and supports the chemisorption-

type immobilization process discussed above. The difference in the sensitivity between the covalently bonded sensors prepared by different methods (Fig. 1) is probably due to a slight difference in α and/or ϕ. On the other hand, the amount of GOD in the crosslinked membrane was estimated to be 3×10^{15} molecules$\cdot$cm^{-2}, or about 150-fold larger than in covalent immobilization. However, the observed sensitivity of the crosslinked membrane sensor is higher than that of the silane coupler method sensor by a factor of only 30. According to Eq. 1, it is expected that a large amount of the immobilized enzyme gives higher sensitivity. However, a too large amount of the immobilized enzyme lowers ϕ, as long as α is constant, and yields an unexpectedly low sensitivity. Thus, assuming a common value of α for all the sensors, we could conclude that about 80% of GOD molecules in the crosslinked membrane do not contribute to the sensor output. To fabricate sensors with high sensitivity, the estimation and control of the aforementioned factors are important.

The results obtained in this work demonstrate the advantage of SnO_2 electrodes for biosensor construction. We are now attempting to fabricate biosensors through further molecular-level surface design of SnO_2 electrodes. As SnO_2 has another feature of optical transparency, the state of surface molecules could be characterized by spectroscopic methods for further improvement. In addition, the semiconducting nature of SnO_2 facilitates fabrication of field effect mode sensors as reported in our separate work.[14)]

This work has been supported partly by a Grant-in-Aid (No. 61227006) from the Ministry of Education, Science and Culture.

References

1) G. G. Guilbault and G. J. Lubrano, Anal. Chim. Acta, 64, 439 (1973).
2) T. Yao, Anal. Chim. Acta, 148, 27 (1983).
3) G. J. Moody, G. S. Sanghera, and J. D. R. Thomas, Analyst (London), 111, 1235 (1986).
4) Y. Onoue and T. Moriizumi, Trans. I.E.E. Jpn. A, 107, 97 (1987).
5) T. Watanabe and H. Gerischer, J. Electroanal. Chem., 117, 185 (1981).
6) P. R. Moses, L. Wiler, and R. W. Murray, Anal. Chem., 47, 1882 (1975).
7) T. Osa and M. Fujihira, Nature , 264, 349 (1976).
8) R. W. Murray, Acc. Chem. Res., 13, 135 (1980).
9) M. Nakao, T. Watanabe, K. Itoh, A. Fujishima, and K. Honda, Ber. Bunsenges. Phys. Chem., 88, 17 (1984).
10) One unit (1 U) of GOD oxidizes 1.0 μmol of β-D-glucose to D-gluconic acid and H_2O_2 per minute.
11) R. M. Ianniello and A. M. Yacynych, Anal. Chem., 53, 2090 (1981).
12) M. Masoom and A. Townshend, Anal. Chim. Acta, 166, 111 (1984).
13) GOD-modified SnO_2 plates were treated with 6 M HCl for 16 h at 110 °C to remove and hydrolyze the immobilized enzymes. The amino acids thus liberated were then determined colorimetrically by the ninhydrin reaction method.
14) Y. Okawa, M. Sukigara, S. Yoshida, and T. Watanabe, Bull. Chem. Soc. Jpn., 61, 1175 (1988).

(Received January 16, 1988)