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Glucose Sensitivity of Poly(mercapto-p-benzoquinone) Films Containing Immobilized Glucose Oxidase

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Glucose oxidase was immobilized in poly(mercapto-p-benzoquinone) film by an electropolymerization of mercaptohydroquinone in the presence of the enzyme. The resulting polymer-coated electrode functioned well as a glucose sensor in an N_2 atmosphere. The sensor showed excellent selectivity for glucose and was possessed of good durability.

Glucose oxidase (GOD) dose not directly transfer electrons to conventional electrodes because the distance between its redox centers and electrode surface is too long even at the nearest distance for electrons to transfer at sufficient rates. Therefore, electrochemical response between the redox centers, oxidized flavin adenine dinucleotides (FAD), of this enzyme and electrodes required the presence of O_2 and H_2O_2,[1] the presence of appropriate electron transfer mediators,[2] or the immobilization of the enzyme on electrodes by using a variety of techniques.[3] Recently, electrochemical immobilization of GOD in conducting polymers such as polypyrrole or polyaniline has been carried out by electropolymerization of the monomers in the presence of GOD.[4] However, the resulting polymer films usually have low sensitivities to glucose. In a previous paper,[5] we reported the preparation of poly(hydroquinone/p-benzoquinone) film (SQ film), a conductive redox polymer, by electropolymerization of mercaptohydro-

quinone. We report here an amperometric glucose sensor, based on direct electrical response between the FAD of GOD and a substrate electrode via the conductive polymer. Immobilization of GOD in the polymer film was carried out by electropolymerization of mercaptohydroquinone in the presence of GOD. The electropolymerization was accomplished at 0.5 V vs. Ag/AgCl for 1 h in a 1 cm^3 phosphate buffer solution (pH 5.6) containing 5 mM mercaptohydroquinone (1M = 1 mol dm^{-3}) and 60 mg GOD (Aspergillus nigar, 15000 — 20000 Ug^{-1}), using a glassy carbon disk electrode (GCE) (3 mm diameter, Furuuchi Kagaku) as a substrate. The resulting electrode (GOD/SQ/GCE) was covered with a dialysis membrane. Electrochemical measurements were performed at 20 °C in an N_2 atmosphere using a three-compartment cell equipped with the GOD/SQ/GCE working electrode, a Pt wire counter electrode, and an Ag/AgCl reference electrode. Figure 1 shows voltammograms for the GOD/SQ/GCE in the presence and in the absence of glucose in the oxygen-free phosphate buffer solution. When glucose was added to the buffer, anodic currents increased with increasing the concentration of glucose. Figure 2 shows current responses of the GOD/SQ/GCE at 0.3 V to glucose. The time required to reach the steady-state current was

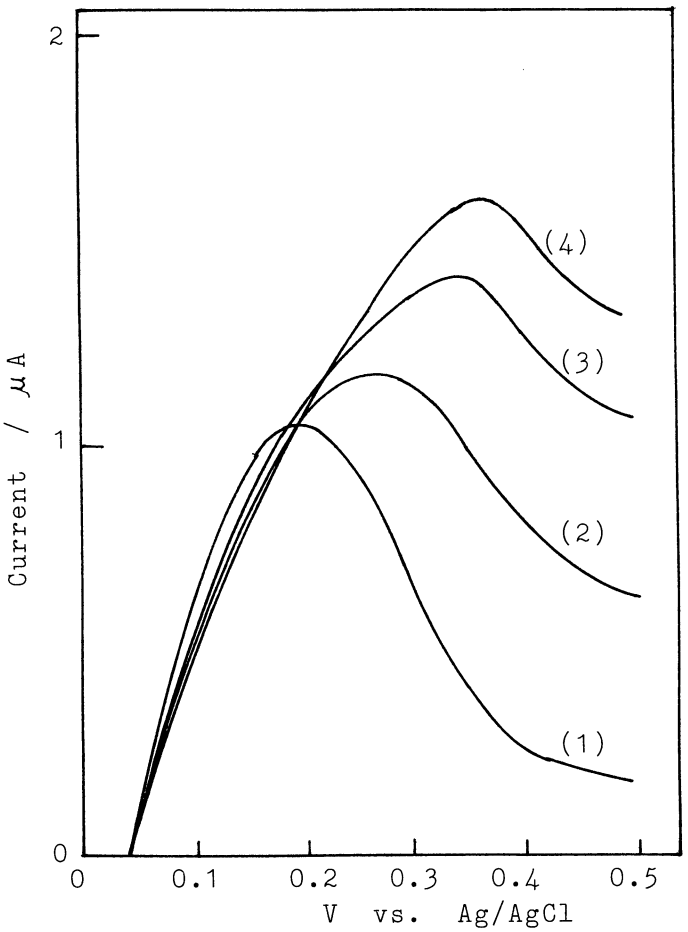

Fig. 1. Oxidation currents of glucose on the GOD/SQ/GCE in a pH 5.6 phosphate buffer solution at a scan rate of 1 mVs^{-1}. Wave(1) shows oxidation current of SQ film. Glucose : (1) 0, (2) 10 mM, (3) 30 mM, (4) 50 mM.

less than 10 s. No appreciable difference in the current response was observed irrespective of the presence of atmospheric oxygen or dissolved oxygen. Typical values for the variation of the steady-state current density (at +0.3 V) with glucose concentration were shown in Fig. 3. The calibration curve of glucose was approximately linear up to 20 mM. In addition, the electrode did not respond to other sugars such as galactose, xylose, arabinose, lactose, mannose, fructose, trehalose, sorbitol, and saccharose. This excellent selectivity implies that the GOD immobilized in the SQ film retains much of its native selectivity and activity, and that

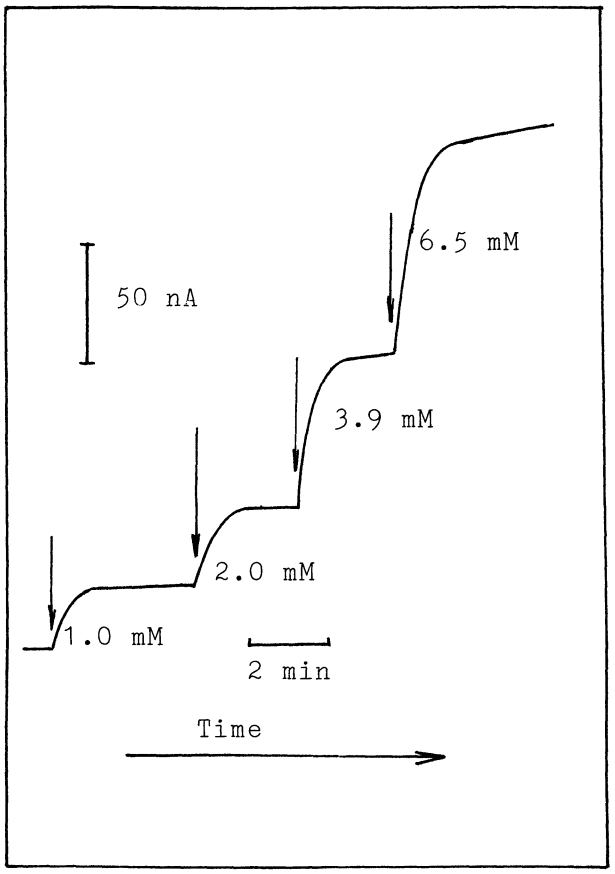

Fig. 2. Increase in oxidation current on the GOD/SQ/GCE with the addition of glucose. Current responses were obtained at a constant potential of 0.3 V at 20 °C using the phosphate buffer solution in N_2 atmosphere. Each arrow indicates the time when 100 mM glucose solution was injected into the test solution.

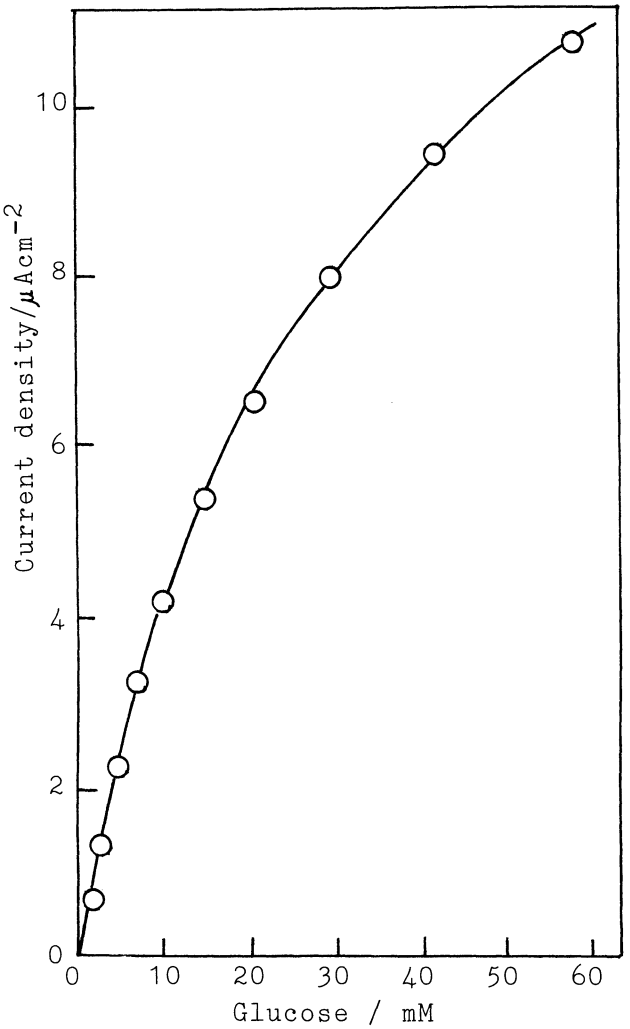

Fig. 3. Calibration curve of glucose. Measurements were carried out under the same conditions as in Fig. 2.

the enzyme conformation is scarcely changed by the fixation in the film. It is difficuly to consider that the mercaptohydroquinone incorporated in the GOD serves an electron-transfer mediator, because the mediator may be attached to the quinone ring of the polymer chain as in the case of the preparation of the SQ film.[6] It may, therefore, be said that the observed glucose sensitivity of the GOD/SQ/GCE must appear as a result of electron -transfer between the quinone moiety in the polymer and the redox center where the polymer serves as an effective electron-transfer chain. When the GOD/SQ/GCE was stored in the phosphate buffer at 25 °C, the current response density decreased by less than 10% even after two weeks. We are presently investgating methods for increasing the stability of the electrode.

References

1) S. J. Updike and G. P. Hicks, Nature, 214, 986 (1971); G. Jonsson and L. Gorton, Anal. Lett., 20, 839 (1987).

2) Y. Kajiya, H.Sugai, C.Iwakura, and H. Yoneyama, Anal. Chem., 63, 49 (1991); P. D. Hale, L. I. Boguslavsky, T. Inagaki, H. I. Karan, H. S. Lee, T. A. Skotheim, and Y. Okamoto, ibid., 63, 677 (1991); B. A. Gregg and A. Heller, J. Phys. Chem., 95, 5976 (1991).

3) T. Ikeda, H. Hamada, and M. Senda, Agric. Biol. Chem., 50, 883 (1986); M. V. Pishko, I. Katakis, S. F. Lindquist, L. Y. Brian, A. Gregg, and A. Heller, Angew. Chem. Int. Ed. Engl., 29, 82 (1990).

4) S. Yabuki, H. Shinohara, and M. Aizawa, J. Chem. Soc., Chem. Commun., 1989, 945; Y. Degani and A. Heller, J. Am. Chem. Soc., 111, 2375 (1989); P. T. Poet, S. Miyamoto, T. Murakami, J. Kimura, and I. Karube, Anal. Chim. Acta, 235, 255 (1990).

5) G. Arai and M. Furui, Nippon Kagaku Kaishi, 1984, 673.

6) The polymerization proceeds alternately by the addition of mercaptohydroquinone in the buffer to quinone ring in the film and the electrooxidation of the attached mercaptohydroquinone to mercapto-p-benzoquinone.

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