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A Novel Glucose Sensor with a Glucose Oxidase Monolayer Immobilized by the Langmuir-Blodgett Technique

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A monomolecular layer of glucose oxidase (GOD) was immobilized on an SnO_2 electrode via glutaraldehyde-bridged covalent bonding of GOD to the amino moieties of an octadecylamine Langmuir-Blodgett film. The performance of the electrode as an amperometric glucose sensor was investigated.

In a recent communication, we reported fabrication of an amperometric glucose sensor by chemical modification of an SnO_2 electrode surface with a monomolecular layer of glucose oxidase (GOD, EC 1.1.3.4).[1] The glucose sensor thus prepared showed a sufficiently high sensitivity, and a more effective contribution of enzymatic process to the sensor response and a wider dynamic range than a conventional type sensor carrying a crosslinked GOD membrane. The Langmuir-Blodgett (LB) technique is an eligible alternative for the molecular-level tailoring of solid electrode surfaces,[2] in that it can introduce functional groups on an electrode with controlled surface orientation and density. This trait has been applied here for the first time to the construction of an enzyme biosensor, through chemical bond formation between a functional LB film and GOD molecules.

Amphiphilic octadecylamine was employed to form a functional spacer LB film for enzyme immobilization. The physical stability of the octadecylamine single monolayer, however, is not sufficient, as judged from a gradual surface pressure lowering at a constant surface area. Mixing the amine with a fairly large amount of octadecanol proved to yield an LB film with improved stability. Covalent bonding of GOD molecules to the spacer LB film on the electrode surface was carried out in the following manner. A monomolecular film of the amphiphiles was spread on a water subphase, and then one monolayer of the film was deposited onto an SnO_2 electrode (Nippon Sheet Glass), pretreated with a 10% toluene solution of trimethylchlorosilane for hydrophobication, at a surface pressure of 20 $mN \cdot m^{-1}$ by a horizontal lifting method with a Kyowa Kaimenkagaku Langmuir trough. The transferred LB monomolecular film on the electrode was treated with a 2.5% glutaraldehyde aqueous solution for 1 h to introduce formyl groups to the outermost

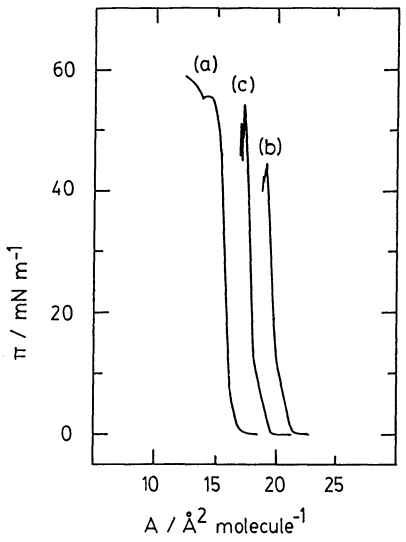

Fig. 1. Surface pressure-area isotherms of octadecylamine (a), octadecanol (b), and octadecylamine/octadecanol (1/10) mixture (c) on a water subphase at 293 K.

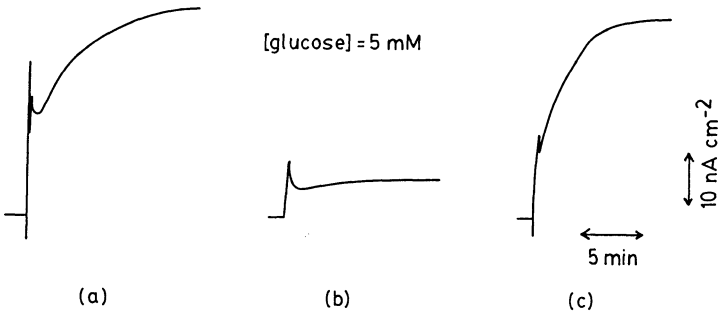

Fig. 2. Response of GOD monolayer electrodes with underlying octadecylamine (a), octadecanol (b), and the amine/alcohol mixture (c) monolayers.

layer. The surface formyl groups were then allowed to react with GOD (Boehringer Mannheim GmbH, 200 $U \cdot mg^{-1}$, 1 $mg \cdot cm^{-3}$ in a pH 7.2 phosphate buffer solution) for 1 h[3] to obtain a GOD monolayer. The sensor performance was evaluated as follows. Using the GOD monolayer-carrying SnO_2 plate as a working electrode, the oxidation current of enzymatically formed H_2O_2 was measured in an O_2-saturated phosphate buffer (pH 6.4) at an electrode potential of 0.85 V vs. Ag/AgCl[4] with a Toho Technical Research potentiostat Model 2020 and a Pt-black counter electrode at 303 K. A stock solution of D-glucose (1 $mol \cdot dm^{-3}$) was allowed to mutarotate for at least 24 h before use.

The sensor response (ΔI) was determined as $I-I_0$, from the background current (I_0) and the steady-state current (I) after the addition of D-glucose solution.

Figure 1 shows surface pressure-area isotherms for octadecylamine, octadecanol, and an octadecylamine/octadecanol mixture (molar ratio 1:10) spread on a water subphase at 293 K (compression rate, 2.5 $Å^2$ $molecule^{-1} \cdot min^{-1}$). The apparent limiting area of mixture film is 19 $Å^2$. This value lies between the limiting area of octadecylamine (17 $Å^2$) and octadecanol (21 $Å^2$), but is smaller than the value predicted from the mixing ratio. This probably reflects the occurrence of an attractive interaction between the amine and the alcohol, and hence a good dispersion of the former in the latter matrix.

Figure 2 depicts the sensor response of GOD monolayer electrodes with three different spacer films, at a D-glucose concentration of 5 $mmol \cdot dm^{-3}$. The LB enzyme layers are stable and neither enzyme desorption nor film detachment was observed during the experiments. The H_2O_2 oxidation current of the electrode with an

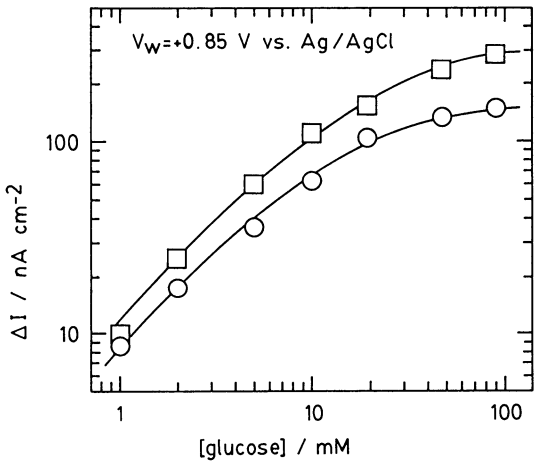

Fig. 3. Current increase (ΔI) vs. glucose concentration profile for the sensors with a GOD monolayer prepared by the LB technique (○) and by chemical modification (□).

Table 1. Substrate specificity of the GOD-LB film sensor for several sugars at 5 mmol·dm^{-3}, pH 6.4, and 303 K

Sugar	Response / nA·cm^{-2}	Relative response
D-Glucose	35.7	100 (standard)
D-Galactose	0	0
D-Fructose	0.1	0.3
D-Mannose	0.3	0.8
L-Glucose	0	0
Sucrose	0.1	0.3

underlying octadecanol monomolecular film (Fig. 2(b)) is markedly smaller than those with underlying LB films containing octadecylamine (Figs. 2(a) and (c)). Since a hydroxyl group cannot react with glutaraldehyde, the response of the electrode with an octadecanol film is due to a small amount of GOD physically entrapped in or adsorbed on the LB film. The response of the electrode with the amine/alcohol mixed film (Fig. 2(c)) is comparable to that with the pure amine film. The matrix alcohol molecules do not interfere with the reaction of the surface amino groups, and one or more amine molecules exist in the cross-sectional area of a GOD molecule on the electrode surface coated with the amine/alcohol mixed film. Thus the amino group surface density on the mixed LB film is sufficient to fill the electrode surface with GOD, and the use of octadecanol as the matrix stabilizes successfully the amine monomolecular layer. The response of these sensors is rather slow, probably because of the limited rate of H_2O_2 permeation through the underlying LB layer. However, further design of the underlying layer could overcome this disadvantage.

The sensor response of the electrode with the amine/alcohol mixed film (○) are plotted as a function of D-glucose concentration in Fig. 3. The sensor exhibits good sensitivity to D-glucose in a concentration range from 1 to 20 mmol·dm^{-3}. The sensitivity of this GOD-LB film electrode is comparable to that of an electrode with GOD covalently immobilized via 3-aminopropyltriethoxysilane and

glutaraldehyde as developed in our previous work[1] (□). Table 1 shows the responses of the GOD-LB film sensor to several sugars under identical conditions as in the above experiment. It is seen that the enzyme immobilization with this LB technique did not lose the substrate specificity of GOD molecules.

This LB technique allows construction of biosensors in which the state of enzyme on the electrode surface is fairly well controlled in contrast to conventional enzyme immobilization methods.[5-9] Using an appropriate functional molecule for the component of the underlying layer, we could construct highly organized assemblies on electrodes, in which the function can be artificially designed on a molecular level. Further works are currently under way toward this end.

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