CHEMISTRY LETTERS, pp. 627-630, 1990.

Amperometric Glucose Sensor with Glucose Oxidase Immobilized on SnO_2 Electrode via a Monolayer of a Photoreactive Nitrophenylazide Derivative

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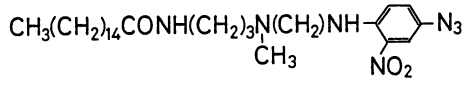
Glucose oxidase (GOD) was immobilized on an SnO_2 electrode via a monolayer of an amphiphilic nitrophenylazide derivative through its photochemical linking reaction. Amperometric glucose sensors with varied electrode-GOD distances were fabricated by the Langmuir-Blodgett technique to assess an efficiency factor in the electrochemical detection of the enzymatic reaction.

Stable immobilization of enzymes and other bioactive proteins in an ultrathin membrane has been the subject of insense study in view of the construction of biofunctional devices. We recently synthesized a novel photoreactive amphiphile, N-(4-azido-2-nitrophenyl)-N'-(N-hexadecanoylaminopropyl)-N'-methyl-1,3-propanediamine, which forms a Langmuir-Blodgett film of a well-ordered structure.[1] This amphiphilic nitrophenylazide (ANPA) acts as a photosensitive precursor for a reactive nitrene radical intermediate which is capable of covalently binding protein molecules, as its analogue has been used as a photolabelling agent.[2] In this study, enzyme immobilization on an ordered array of such a reactive amphiphile was applied for the first time to the fabrication of biosensors. This communication discloses the performance of the enzyme-bound monolayer-coated glucose sensor prepared by a rapid single-step binding reaction of this unique amphiphile.



ANPA

The monolayer of ANPA was prepared on a neutral aqueous subphase in a previously described manner.[1] An SnO_2 electrode (Nippon Sheet Glass Co., 4500 Å thick SnO_2 layer on glass, specific resistance 0.0004 ohm·cm) was rendered hydrophobic by treating it with a 10% toluene solution of trichloromethylsilane for 1 h at 60 °C, the completion of silanization being checked by XPS.

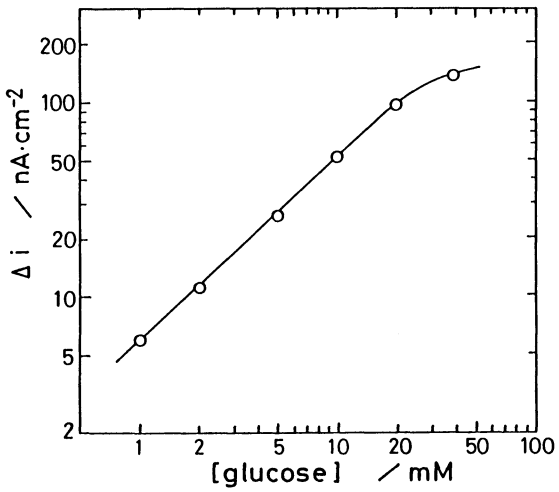


Fig. 1. Sensor response as a function of glucose concentration. Electrolyte, 1/15 M phosphate buffer (pH 6.4); electrode potential, + 1.2 V vs. SCE. Increase in anodic current (Δ i) caused by glucose addition was measured 3 min after the addition under non-stirred condition at room temperature.

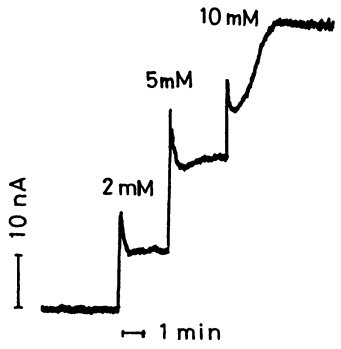


Fig. 2. Current response profile of the sensor in the course of a stepwise addition of glucose. The solution was temporarily stirred on glucose addition and left under non-stirred condition to monitor a steady state current as a sonsor output.

A monolayer of ANPA was deposited on the SnO_2 electrode by the horizontal lifting method under a surface pressure of 20 $mN \cdot m^{-1}$. For samples having the underlying spacer layers, arachidic acid monolayers were built up on SnO_2 prior to the coating of ANPA. Glucose oxidase (GOD, Boehringer Mannheim, Grade II) was dissolved in a 1/15 M (1 M = 1 $mol \cdot dm^{-3}$) phosphate buffer solution (pH 6.4) at a concentration of 10^{-5} M. The ANPA monolayer on the SnO_2 electrode was left in contact with the GOD solution for about 1 h at room temperature and then exposed to visible light for 2 min by a 150 W xenon arc lamp to complete the photolysis of ANPA.[1] After repeated rinsing, the GOD-immobilized SnO_2 electrode thus prepared was set in an electrochemical cell together with a saturated calomel reference electrode (SCE) and a platinum counter electrode, the 1/15 M phosphate buffer being used as an electrolyte solution to which glucose is to be added as a substrate.

The electrochemical measurement for sensing glucose was based on the method of Watanabe et al.[3] or Tsuzuki et al.[4] A concentrated glucose solution (1 M) was added to the electrolyte (ca. 60 cm^3) to measure the sensor response. The response was monitored as an increase in the anodic current (Δ i) caused by the oxidation of H_2O_2 which is produced through the enzymatic oxidation reaction of glucose in the presence of oxygen. The electrode potential for sensing was set at + 1.2 V vs. SCE by means of a Toho Technical Research potentiostat Model 2020.

Figure 1 exhibits a sensor output as a function of the glucose

concentration. The sensor showed a good linearity of response against the concentrations up to about 20 mM. On addition of 20 mM glucose, an output current of the order of 10^{-7} A·cm^{-2} was normally attained. Figure 2 dipicts a typical current response to a stepwise addition of glucose. The response time was within 1 min at relatively low concentration of glucose ($\leq$ 5 mM) and tended to be longer at the higher concentration. Oxygen bubbling into the electrolyte largely recovered the output and improved the response time in the non-linear region (> 20 mM). The response time obtained by this sensor is definitely shorter than those which have been reported with thicker matrices of enzymes such as a Langmuir-Blodgett multilayer[5] and polymer gels[6,7] and is comparable with those obtained for other monolayer-thick membranes.[3,4,8,9]

In addition to the rapidity of response, a high detection sensitivity, i. e., high output current is required for a sensor. Of primary importance in this context is to obtain a high surface concentration of the enzyme immobilized as a monolayer and to attain a high efficiency for the amperometric detection of enzymatic reaction. Our investigation with ^{125}I-labelled GOD revealed that the surface concentration of GOD immobilized on the ANPA monolayer attained a close-packed monolayer coverage of this enzyme. The enzymatic activity per unit area of this system was measured to be about 1.5×10^{-11} mol·s^{-1}cm^{-2} at 10 mM of glucose which corresponds to a GOD specific activity (turnover number) of about 8 s^{-1}. The activity per area value (a) allows us to estimate the efficiency factor (f) for the electrochemical capture of the enzymatic reaction product (H_2O_2) according to the equation;

$$f = \Delta i / (a \cdot n \cdot F)$$

Here, Δ i denotes the current density of response obtained under the same condition as in the activity measurement. Symbols n and f represent charge number (= 2) and the Faraday constant, respectively. Using the Δ i value of 50 nA·cm^{-2} at 10 mM glucose, we obtain an f value for the present sensor system to be on the order of 2×10^{-2}, indicating that only a small portion of the product can be captured by the electrode reaction.

This efficiency is, however, further reduced for the enzyme molecules present in/on a layer

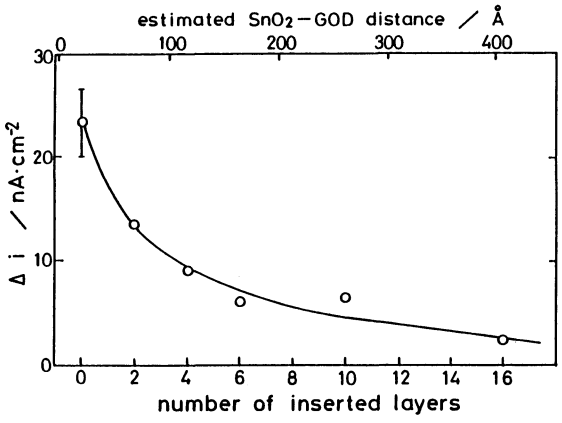


Fig. 3. Plot of Δ i against the number of arachidic acid monolayers inserted between SnO_2 and GOD molecules or the estimated distance between them based on the monolayer thickness (24 Å / layer). The current was measured at 5 mM of added glucose.

which is separated by several monolayers from the electrode surface. This phenomenon is demonstrated in Fig. 3, where the top ANPA monolayer bearing GOD was separated from SnO_2 by the inserted monolayers of arachidic acid. Obviously, a rapid decrease in response took place with an increase in the distance between the electrode and the enzyme. The result can be interpreted as a suppression effect of the intermediate layer on the diffusion transfer of H_2O_2 to the electrode surface and is an indication that a large loss in sensing efficiency is inevitably involved in a thick membrane of more than several monolayers with its degree depending on the nature of the medium. For comparison, a preliminary experiment was undertaken in which GOD was immobilized on an SnO_2 electrode by use of bovine serum albumin crossliked with gultaraldehyde as a binder medium. This gave a sensor membrane with an estimated GOD amount of more than 40 monolayers. The response obtained with this system was about 10-fold larger than the value obtained here, indicating that the monolayer system is efficient by about 4-fold relative to the thick crosslinked system.

Use of an ultrathin sensor membrane with high surface concentration of enzyme is essential for attaining a rapid response as well as a desired sensitivity for the amperometric detection. The sensor developed here is expected to meet this end and is thus promising. In order to fabricate a potentiometric sensor which permits a micron-order miniaturization of an electrode for effective sensing, a field effect transistor is also a useful candidate for the substrate of the present sensor membrane.

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(Received January 12, 1990)