Photo-switchable Ion and Enzyme Sensors. Photoinduced Potentiometric Response of Glassy Carbon Electrode Coated with Polymer or Polymer/Enzyme Dual Membrane

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Photo-switchable ion and enzyme sensors were fabricated by the use of glassy carbon electrode coated with nonactin-doped or enzyme modified poly(vinyl chloride) (PVC) membranes. The ion sensor with nonactin-doped PVC membrane, which contained spirobenzopyran as the photosensitive dye, exhibited a potentiometric photoresponse to NH_4^+ ion in the solution. The dynamic range of the NH_4^+ ion sensor was 10^{-7}—10^{-3} M. Urea, adenosine, and asparagine sensors were prepared by coating the surface of the NH_4^+-ion sensor with urease, adenosine deaminase, and asparaginase membranes, respectively. These enzyme sensors could be used for determining the substrates at the micro mole level. The performance characteristics of these sensors were compared with those previously prepared membrane electrode sensors.

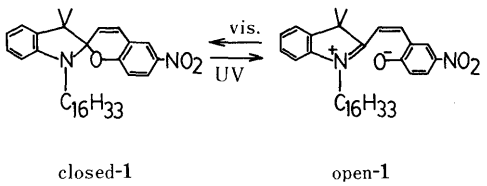
Keywords ion sensor; enzyme sensor; photoresponse; spirobenzopyran; glassy carbon electrode; urease; adenosine deaminase; asparaginase

We have developed a novel class of ion and enzyme sensors which can be switched on and off by light signal by the use of photosensitive membranes composed of poly(vinyl chloride) (PVC) and a spirobenzopyran derivative (**1**) (Fig. 1).[1-5] The principle of operation of the sensors is that the photoinduced membrane potential across the membrane can be modulated by the specific adsorption of ions on the membrane surface or by the enzymatic reactions occurring on the membrane surface. These sensors have been fabricated from U-shaped glass cells in which the membrane separates two electolyte solutions, *i.e.*, internal reference solution and sample solution (we designate such a system as a "membrane electrode" in the present paper).[1-4] On the other hand, the present paper describes the preparation of coated-wire electrode sensors for NH_4^+ ion and urea, adenosine and asparagine. This approach is useful for developing probe type and even miniature sensors.

In comparison with conventional types of ion and enzyme sensors, the photo-switchable sensors are characterized by a higher sensitivity to low concentrations of substrates than to high concentrations, though the extra procedure of ultraviolet (UV) irradiation is necessary to measure the potential change. The output signal of the sensors is stable during light irradiation.

Experimental

Materials PVC(polymerization degree 1100) was purchased from Wako Co., Ltd. and used without further purification. Dibutyl phthalate (DBP), tetrahydrofuran (THF), urea, adenosine, asparagine, glutaraldehyde, and nonactin were of extra pure reagent grade. Urease (EC 3.5.1.5), adenosine deaminase (EC 3.5.4.4, Type 2) and bovine serum albumin (BSA, crystallized and lyophilized powder) were purchased from Sigma Co. Asparaginase (EC 3.5.1.1) was obtained from Kyowa Hakko Co. The synthetic procedure and analytical data for **1** were reported previously.[6] Glassy carbon (GC) rod was obtained from Tokai Carbon Co.



closed-**1** open-**1**

Fig. 1. Photochemical Reaction of Spirobenzopyran **1**

Sensor Fabrication The structure of the sensor is illustrated in Fig. 2. The top of a GC rod (3 mm diameter) mounted in a Teflon rod was coated with the photosensitive polymer membrane composed of PVC (31%), DBP (63%), nonactin (4%), and **1** (2%). The polymer membrane was prepared by pouring a small amount of THF solution of the materials onto the surface of the GC electrode and allowing the solvent to evaporate. The thickness of the membrane thus prepared was *ca.* 0.1 mm. For the preparation of enzyme sensors, the polymer layer of the electrode was further covered with an enzyme membrane, which was prepared from a mixture composed of equal amounts of 10% enzyme solution, 10% BSA solution, and 8% glutaraldehyde. After evaporation of the water, a thin layer of immobilized-enzyme membrane was formed on the electrode. The

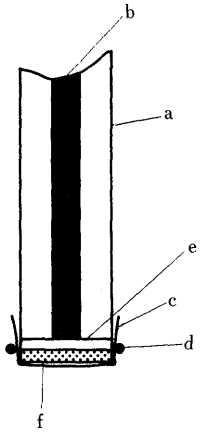


Fig. 2. Schematic Representation of the Sensor

a, Teflon rod; b, GC rod; c, Nylon mesh; d, O-ring; e, PVC/**1**/nonactin membrane; f, enzyme layer.

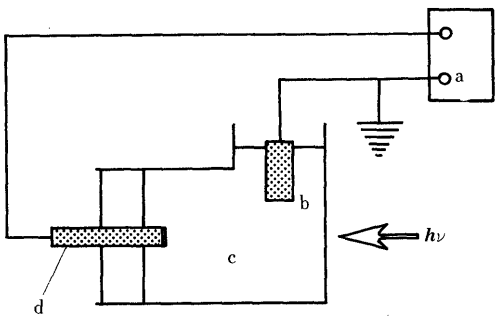


Fig. 3. Schematic Representation of the Cell for the Measurement of Potentiometric Response

a, potentiometer; b, reference electrode; c, sample solution; d, sensor.

thickness of the enzyme membrane was *ca.* 5 μm. The sensitive layer was wrapped with nylon mesh.

Measurement All potentiometric measurements were carried out at 23 °C, using a glass cell (Fig. 3). The electrochemical cell was as follows: GC electrode | PVC/**1**/nonactin membrane | enzyme layer | sample solution | reference electrode (0.1 M $(CH_3)_4NCl$ | Ag/AgCl). The pH of the solution was regulated with a modified Britton–Robinson buffer (LiOH was used in place of NaOH). The light source was a 500 W xenon lamp and cut-off filters (Toshiba UVD-35 and O-55) were used for isolating UV (320 nm $< \lambda <$ 400 nm) and visible ($\lambda >$ 550 nm) light, respectively. Noise level was within 1 mV under the present experimental conditions.

Results and Discussion

The potentiometric photoresponse of the GC electrode coated with a PVC/**1**/nonactin membrane was examined (Fig. 4). After the steady-state potential had been obtained in the dark, the electrode potential shifted rapidly in the positive direction upon UV light irradiation, and reached a steady-state value in *ca.* 5 min. The visible light irradiation restored the original potential. The photoresponsive behavior of the electrode potential is basically the same as that observed for a membrane electrode separating two aqueous phases.[1-5] The surface potential change of the PVC/**1**/nonactin membrane, which stems from the formation of open-**1**, should be responsible for the potentiometric response. The photochemical formation of open-**1** can be ascertained visually, as a color change of the membrane to deep purple. The mechanism by which the modified GC electrode generated photopotential does not involve ion permeation across the membrane, because one side of the membrane is blocked so as to inhibit transfer of ions by the GC surface in the present system. In other words, those results strongly support our previous explanation that the potentiometric photoresponse of the **1**-doped membrane results from the change in surface potential, not in diffusion potential.[1-5] Figure 5 plots the magnitude of photoinduced potential of the PVC/**1**/nonactin membrane-coated GC electrode against the concentration of NH_4Cl in the solution. The magnitude of photoinduced potential, $\Delta(\Delta\phi)$, decreased with increasing concentration of NH_4Cl, and, above 10^{-3} M NH_4Cl, the $\Delta(\Delta\phi)$ values were suppressed to below 10 mV. These results can be explained in terms of the specific adsorption of NH_4^+ ions on the membrane surface by forming complexes with nonactin, as already discussed in detail for the photoresponse of PVC/**1**/nonactin membrane in the presence of NH_4Cl.[1] From the viewpoint of sensor application of the modified GC electrode, it may be possible to determine NH_4^+ ion concentration at a μM level. Figure 5 also shows the photoresponse of the electrode coated with a

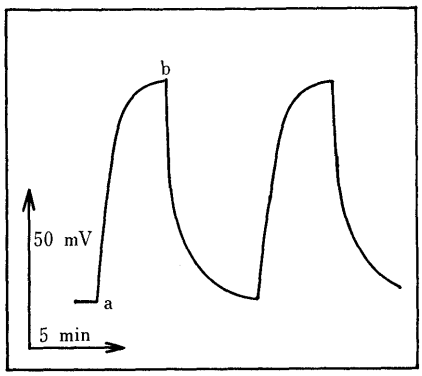


Fig. 4. Typical Photoresponse of the PVC/**1**/Nonactin Membrane-Coated GC Electrode in the Presence of 10^{-5} M NH_4Cl at pH 6

a: UV light irradiation, b: visible light irradiation.

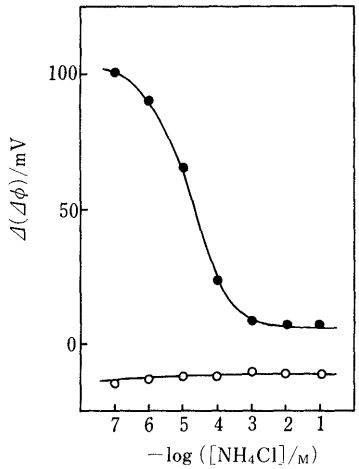


Fig. 5. Effects of NH_4Cl Concentration on the Magnitude of Photoresponse of the PVC/**1**/Nonactin or PVC/**1** Membrane-Coated GC Electrode

Photoresponse of PVC/**1**/nonactin (—●—) and PVC/**1** membrane-coated (—○—) electrodes.

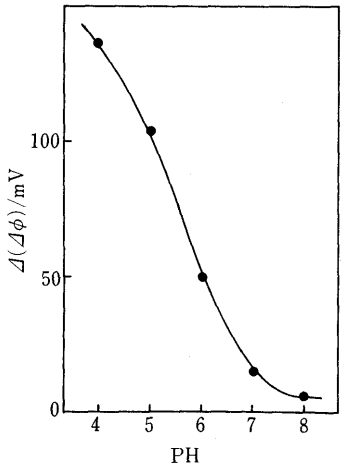


Fig. 6. Effects of pH on the Magnitude of Photoresponse of the PVC/**1**/Nonactin Membrane-Coated GC Electrode

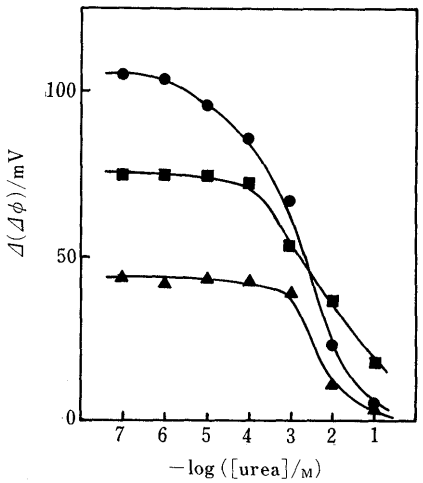


Fig. 7. Typical Calibration Graphs of the Urea Sensor at pH 6.0

The buffer concentration was 10 (—●—), 40 (—■—), or 100 mM (—▲—).

spirobenzopyran-free membrane. The $\Delta(\Delta\phi)$ values of *ca.* -10 mV were observed irrespective of the NH_4Cl concentration; the origin of these is rather obscure.

The photoresponse of the PVC/**1**/nonactin-coated GC electrode is sensitive to the pH of the solution. Figure 6 shows the $\Delta(\Delta\phi)$ values measured at pH 5—9. This result is in line with the data previously obtained for the membrane electrode.[1-5] The pH dependent photoresponse of the electrode can be ascribed to the acid-base equilibrium of the open form of **1** at the membrane surface, as described previously.[1-5]

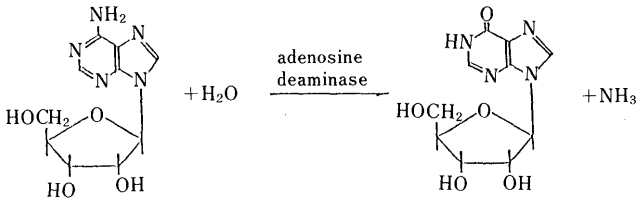
We have prepared small enzyme sensors using the PVC/**1**/coated GC electrode as an underlying electrode. Since the $\Delta(\Delta\phi)$ value of the PVC/**1**/nonactin-coated electrode depends considerably on the concentration of NH_4^+ ion and pH in the solution, the potentiometric photoresponse of the electrode should be modulated if an enzymatic reaction which produces NH_4^+ ion occurs on the electrode surface. From this viewpoint, three kinds of enzymes were immobilized on the surface of the PVC/**1**/nonactin layer of the electrode: urease, asparaginase, and adenosine deaminase.

Figure 7 shows calibration curves of the urea sensor in 10, 40, and 100 mM buffers. In all cases, the $\Delta(\Delta\phi)$ value decreased with increasing concentration of urea in the solution. This tendency can be attributed to the enhanced local concentration of NH_4^+ ion and to the pH change at the surface of the PVC/**1**/nonactin layer of the electrode, as a result of the decomposition reaction of urea catalyzed by urease as follows:

$$(NH_2)_2CO + 2H_2O + H^+ \rightarrow 2NH_4^+ + HCO_3^-$$

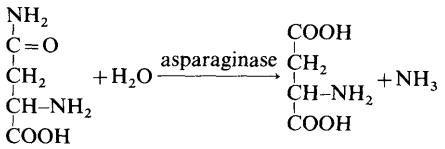
The urea sensor exhibited high sensitivity and a wide usable range of the calibration graph in 10 mM buffer. In contrast, the usable range of the calibration graph is limited to $> 10^{-4}$ M urea in 40 mM buffer. The calibration graph in 100 mM buffer is practically useless. These results may arise from the attenuated photoresponse of the PVC/**1**/nonactin-coated electrode itself, not from deterioration of the enzyme activity. The present system may involve two separate mechanisms by which the photoresponse of the sensor can be affected by the pH of the urea solution. One is the pH-dependent catalytic activity of urease, and the other is the pH dependence of the $\Delta(\Delta\phi)$ value of the PVC/**1**/nonactin-coated electrode itself, which can be seen even in the absence of urea, as shown in Fig. 6. Therefore, the effect of pH of the urea solution upon the photoresponse was examined over the range of pH 5.0—8.0 in the presence of 10^{-7}—10^{-1} M urea (Fig. 8). At pH 5.0 and 6.0 the $\Delta(\Delta\phi)$ values depended clearly on the concentration of urea, which suggests that urease catalyses the decomposition reaction of urea in this pH region. This is quite reasonable judging from the fact that the curve of the urease activity is bell-shaped with the maximum of pH 6.0—7.0.[7] In pH 7.0 and 8.0 media the $\Delta(\Delta\phi)$ values were suppressed considerably even when the concentration of urea was sufficiently low, 10^{-7}—10^{-6} M. The reason for this is that the $\Delta(\Delta\phi)$, irrespective of enzymatic reaction, is intrinsically dependent upon the pH of the solution as shown in Fig. 6. From these data, this urea sensor is recommended to be operated in 10 mM buffer of pH 5—6.

Adenosine deaminase deaminates adenosine to produce inosine and ammonia as follows:



It should be possible to construct an adenosine sensor by modifying the surface of the PVC/**1**/nonactin-coated GC electrode with adenosine deaminase. Figure 9 plots the $\Delta(\Delta\phi)$ values of the adenosine sensor in 10 mM buffer at pH 6. As expected, the $\Delta(\Delta\phi)$ value decreased with increasing concentration of adenosine in the aqueous solution.

An asparagine sensor was also prepared by the use of asparaginase, which catalyzes the decomposition reaction of L-asparagine to L-aspartic acid and ammonia as follows:



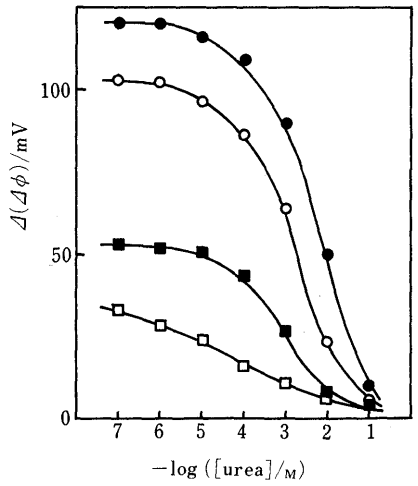


Fig. 8. Effects of Buffer pH on the Photoresponse of the Urea Sensor in 10 mM Buffer

The pH of the buffer was 5.0 (—●—), 6.0 (—○—), 7.0 (—■—), or 8.0 (—□—).

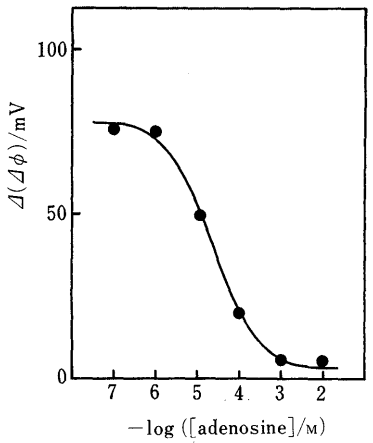


Fig. 9. A Calibration Graph of the Adenosine Sensor

The 10 mM buffer (pH 6.0) was used.

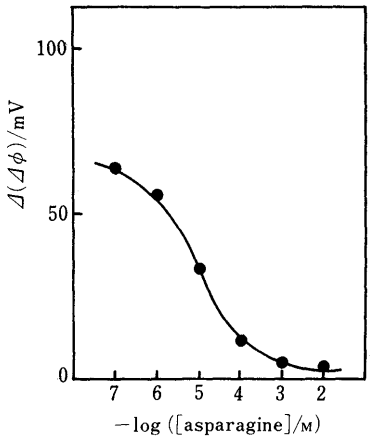


Fig. 10. A Calibration Graph of the Asparagine Sensor

The 10 mM buffer (pH 6.0) was used.

Figure 10 shows the calibration graph of the asparagine sensor in 10 mM buffer at pH 6. The profiles of the calibration graph of the adenosine and asparagine sensors are almost the same as those previously obtained under the same experimental conditions for the sensors based on the membrane electrodes.

The durability of the urea sensor was examined. Figure 11 shows calibration curves obtained at 1, 7, 10, and 14 d after preparation of the sensor. The sensor was stored in the working buffer at 4 °C in a refrigerator when not in use. The data show that the sensor can be used for about 10 d. The limited life time of the sensor is presumably due to the deterioration of **1** and/or urease.

These results show that the use of the PVC/**1**/nonactin-coated GC electrode, in which the internal reference solution is eliminated from the membrane electrode system, introduces no problem in constructing enzyme sensors, although the durability of the sensor needs to be improved. Miniaturization of the sensor body is therefore possible by the use of a microsize GC electrode.

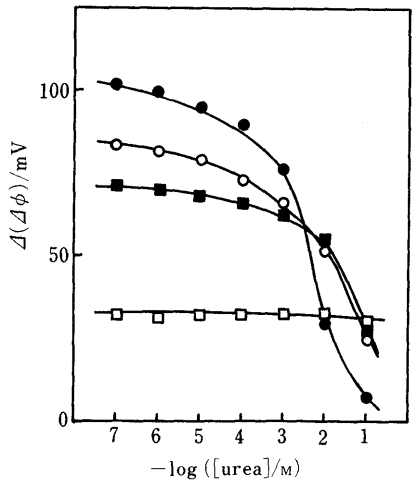


Fig. 11. Durability of the Urea Sensor

Calibration graphs measured at 1 (—●—), 7 (—○—), 10 (—■—), and 14 d (—□—) after sensor preparation.

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