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Enzyme Sensors Based on Coated-Wire Electrode. Use of Carboxyl-Substituted Poly(vinyl chloride) as a Support for Immobilizing Penicillinase

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A poly(vinyl chloride) derivative which contained 1.8% carboxyl residue was used to prepare a potentiometric sensor sensitive to penicillin G. A pH-sensitive base electrode was fabricated by covering an Ag wire electrode with the membrane composed of the carboxyl-substituted poly(vinyl chloride), *o*-nitrophenyl octyl ether, and tridodecylamine. The sensitive layer of the pH-sensitive electrode was modified with penicillinase to make a penicillin-sensitive electrode. Penicillinase was adsorbed to the surface of the polymer membrane by immersing the sensitive layer of the electrode in the penicillinase solution. Treatment of the pH-sensitive electrode with ca. 0.3% penicillinase solution at pH 6.7 (1 mM†† phosphate buffer) resulted in favorable performance of the penicillin sensor. The long-term stability of the penicillin sensor thus prepared was satisfactorily good.

Enzyme sensors have been considered to have great potential as analytical tools for sensing precursors and metabolites in biological fluids, some kinds of drugs, and other biologically important compounds. To data, many kinds of electric and optical devices, including ion- and gas-sensitive conventional types of electrodes, semiconductor electrodes, thermistors, and optical fibers, coupled with enzymes have been employed for developing enzyme sensors.[1-10]

Many researchers have used potentiometric pH-sensitive devices as base electrodes on which enzyme is immobilized. Mosbach et al. reported that a pH glass electrode can be used as a base electrode to construct enzyme sensors sensitive to glucose, urea, and penicillin by covering the electrode surface with glucose oxidase, urease, and penicillinase membranes, respectively.[11] The principle of operation of the sensors was that the changes in H^+ concentration arising from the enzymatic reaction in the enzyme membrane were detected as local pH change by the pH glass electrode.

Recently, metal electrodes coated with metal oxide layer (IrO_2 or RuO_2), which can serve as pH sensors, have been utilized as base electrodes for preparing potentiometric enzyme sensors.[12-15] The metal oxide electrodes are of considerable interest due to their mechanical strength as well as ease of miniaturization as compared with the conventional type of glass electrode.

We have used an ion-sensitive field effect transistor (ISFET) as a pH-sensitive base electrode for developing micro enzyme sensors.[16-19] In the course of our study on the ISFET-based enzyme sensors, it has become obvious that high-performance enzyme sensors can be prepared by covering the ISFET gate with the enzymes which catalyze the reactions resulting in pH changes of the medium. Another interesting device for preparing potentiometric enzyme sensors is a pH-sensitive metal electrode coated with solvent polymeric membrane containing neutral carrier sensitive to H^+. The membrane-coated Ag electrode has a merit in miniaturizing the sensor body due to the absence of internal reference solution. We have already reported that urea and penicillin sensors can be prepared based on the Ag electrode which is coated with poly(vinyl chloride)–tridodecylamine composite membrane coupled with the enzyme–albumin crosslinked membrane.[20,21] However, the life time of the enzyme–albumin crosslinked membrane was not long. A poor surface adhesion between the polymer membrane and the enzyme membrane was also a drawback.

In this paper, we report that the poly(vinyl chloride) derivative which contains 1.8 wt% carboxyl residue can be used as a polymer matrix of pH-sensitive coated-wire electrode and that, using the coated-wire electrode, penicillin-sensitive electrode can be prepared by immobilizing penicillinase on the polymer surface mainly through electrostatic interaction. The present technique would provide a facile method to make enzyme sensors by the use of polymer-coated electrode.

Experimental

Materials. The carboxyl-substituted poly(vinyl chloride) (PVC-COOH), nominally 1.8 wt% carboxyl residue, was used as received from Aldrich Chemical Co. Poly(vinyl chloride) (PVC) was purchased from Wako Chemical Co. Sodium tetraphenylborate (NaTPB), tridodecylamine (TDA), and *o*-nitrophenyl octyl ether (NPOE) were of extra pure reagent grade. Penicillinase was purchased from Miles Chemical Co. Penicillin G potassium salt was obtained from Wako Chemical Co.

Electrode Preparation. Figure 1 shows the schematic representation of the electrode. The top of Ag wire (0.5 mm

†† (M=mol·dm^{-3}).

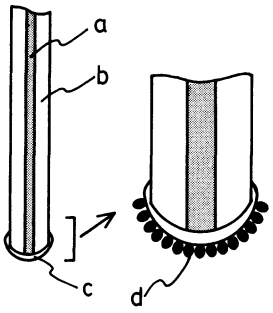


Fig. 1. Schematic representation of the penicillin-sensitive electrode.
a: Ag wire; b: Teflon rod; c: PVC-COOH-TDA membrane; d: penicillinase.

diameter) mounted in a Teflon rod was coated with a pH-sensitive solvent polymeric membrane composed of PVC-COOH (31%), NPOE (63%), TDA (5.5%), and NaTPB (0.5%). The polymer membrane was prepared by dip-coating method using tetrahydrofuran solution of the materials. The thickness of the polymer membrane thus prepared was about 0.2 mm. Prior to enzyme immobilization, the pH response of the coated-wire electrode was checked. Then, the polymer layer of the electrode was further modified with penicillinase by immersing the top of the electrode in the penicillinase solution. After treatment for about 15 h at 5—10 °C, the probe was rinsed adequately with the working buffer.

Measurements. All potentiometric measurements were carried out at 23 °C, using a digital multimeter (TR 6843, Takeda Riken Co.) and a saturated calomel electrode (SCE). Solutions were not stirred during the measurements, and the probe was rinsed with the working buffer after each measurement. The electrode was stored in a refrigerator at about 4 °C when not in use.

Results and Discussion

PVC-TDA-Coated Electrode. It has been demonstrated previously that the PVC–TDA composite membrane can serve as a pH sensor.[22,23] A metal electrode coated with the PVC–TDA membrane was also examined.[24] The use of PVC-COOH for preparing coated-wire electrode has been first reported by Satchwill and Harrison.[25] They have demonstrated that the potentiometric response of K^+-sensitive electrode coated with the PVC-COOH-*valinomycin* membrane is comparable to that of the parent PVC-based device.

The pH response of the PVC-COOH–TDA-coated electrode was checked using 50 mM KH_2PO_4 solutions containing varying amounts of NaOH (Fig. 2). The electrode potential depended linearly on the solution pH over the range of pH 4.3—10.2. The pH sensitivity was about 50 mV/decade, and the response was satisfactorily fast (20—30 s). The pH response of the electrode is almost the same as that of the PVC–TDA-based electrode.[22-24] These results suggest that the PVC-COOH can be used as a polymer matrix of the pH-sensitive membrane for constructing enzyme sensors.

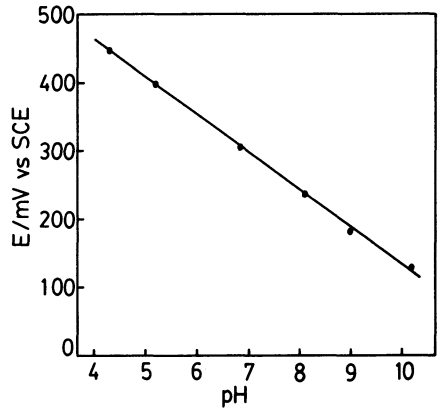


Fig. 2. pH response of the PVC-COOH–TDA-coated electrode. The pH values of the solutions were adjusted by adding varying amounts of NaOH to 50 mM KH_2PO_4.

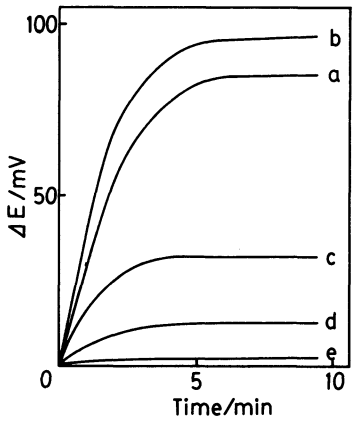


Fig. 3. Typical response curves of the penicillin sensor for penicillin G solutions in 5 mM phosphate buffer at pH 7.0.
Penicillin G concentration: a: 30; b: 10; c: 3; d: 1; e: 0.1 mM.

Penicillin-Sensitive Electrode. The PVC-COOH–TDA-coated electrode was modified with penicillinase for making penicillin-sensitive electrode, by treating the sensitive layer with 0.3% penicillinase solution which was prepared in 1 mM phosphate buffer at pH 7.0. The potentiometric response of the penicillin sensor thus prepared was examined for 0.1—30 mM penicillin G in 5 mM phosphate buffer of pH 7.0 (Figs. 3 and 4). When the electrode was immersed in the penicillin G solution, the electrode potential shifted in the positive direction and reached steady-state value after 4—6 min. The positive shift of the potential means that the pH value around the PVC-COOH–TDA membrane decreased (cf. Fig. 2). This is reasonably explained based on the enzymatic reaction of penicillinase (1), where H^+ is liberated from

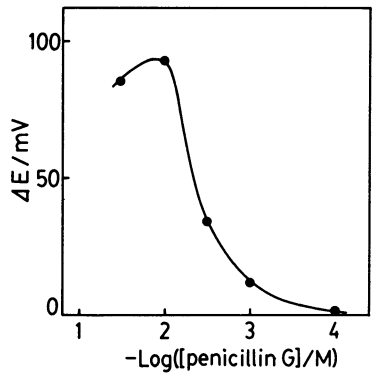


Fig. 4. Calibration graph of the penicillin sensor in 5 mM phosphate buffer at pH 7.0.

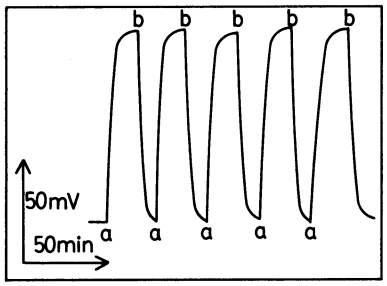


Fig. 5. Reproducibility of the penicillin sensor. Sample solution was 10 mM penicillin G in 5 mM phosphate buffer at pH 7.0. The sensor was immersed in the sample solution (a) and rinsed with the working buffer (b).

penicilloic acid produced. The value of the potential shift, ΔE, depended on the concentration of penicillin G in the solution. It is clear that penicillinase was adsorbed irreversibly to the surface of the PVC-COOH-TDA membrane without inactivation. The reduced response of the sensor for 30 mM penicillin G solution may be explained based on the fact that, as a result of the progress of the enzymatic reaction, the pH value around the polymer surface shifted down to ca. pH 5 at which penicillinase lost its catalytic activity to some degree.[26]

The reproducibility of response of the penicillin sensor was checked by repeatedly immersing the probe in 10 mM penicillin G in 5 mM phosphate buffer at pH 7.0 (Fig. 5). The reproducibility of the sensor was satisfactorily good. After the electrode potential had reached a steady-state value in the sample solution, the original potential was recovered in 10—15 min by immersing the probe in the working buffer. Potential drift during the series of measurements was small.

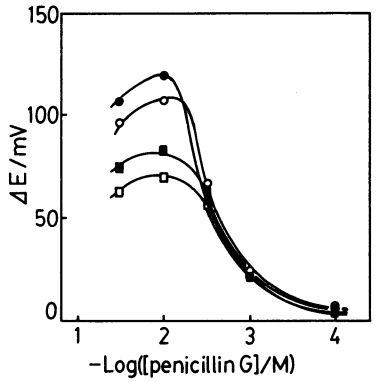
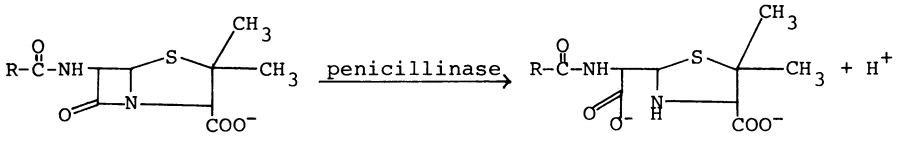


Fig. 6. Effects of pH of penicillinase solution on the response of the sensor.
The pH values of the penicillinase solutions were 5.2 (—□—), 6.0 (—○—), 6.7 (—●—), and 7.8 (—■—). The penicillin G solutions were prepared in 5 mM phosphate buffer at pH 7.0.

We also tried to immobilize penicillinase to the pH-sensitive electrode prepared by the use of parent PVC-TDA membrane under the same conditions. After treatment with penicillinase solution, the PVC-TDA-coated electrode responded to penicillin G to some extent, but the response disappeared after repeated measurements of the sample. This is presumably due to the desorption of penicillinase from the surface of PVC-TDA membrane. These results suggest that the carboxyl groups in PVC-COOH serve as ionic sites for binding penicillinase by the electrostatic force of attraction.

In order to establish the optimum conditions of enzyme solution in which the pH-sensitive electrode is treated, the effects of pH and ionic concentration of the enzyme solution, as well as the enzyme concentration, on the potentiometric response were examined. Figure 6 illustrates the calibration graphs for the enzyme sensors prepared by treating the probe in 0.3% penicillinase solutions at pH 5.2, 6.0, 6.7, and 7.8. At all pH tested, penicillinase was adsorbed to the membrane surface. The penicillin G sensors prepared at pH 6.0 and 6.7 showed relatively higher response as compared with those prepared at pH 5.2 and 7.8. These results imply that the electrode surface binds penicillinase efficiently in the pH region where PVC-COOH and penicillinase are charged negatively and positively, respectively.

Figure 7 shows the effects of buffer strength of the penicillinase solutions in which the PVC-COOH-TDA-coated electrode was treated for immobilizing penicillinase. The concentration of penicillinase was



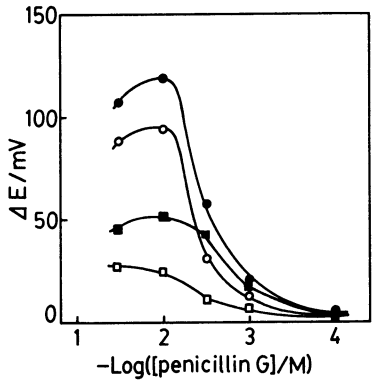


Fig. 7. Effects of buffer strength of penicillinase solution on the response of the sensor.
The buffer concentrations of penicillinase solutions were 1 (—●—), 5 (—○—), 20 (—■—), and 50 mM (—□—).
The penicillin G solutions were the same as used for Fig. 6.

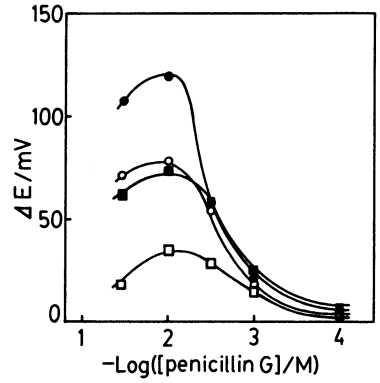


Fig. 8. Effects of penicillinase concentration on the response of the sensor.
The penicillinase concentrations were 0.07% (—■—), 0.3% (—●—), 0.7% (—○—), and 1.3% (—□—).
The penicillinase solutions were prepared in 1 mM phosphate buffer at pH 6.7.
The penicillin G solutions were the same as used for Fig. 6.

0.3% and the pH of the solutions was adjusted at 6.7. The results clearly demonstrate that the penicillin sensors prepared in the lower strength buffers exhibit higher response. This should arise from the difference in the enzyme load on the electrode. In other words, the penicillinase solution of lower buffer concentration favors the binding of penicillinase to the membrane, which situation agrees with the fact that the main force of attraction between penicillinase and PVC-COOH-TDA membrane is electrostatic one. Mitz et al. reported a similar effect of salt concentration, which was observed in immobilizing enzymes onto some cellulose ion exchangers.[27]

In Fig. 8 are shown the effects of penicillinase

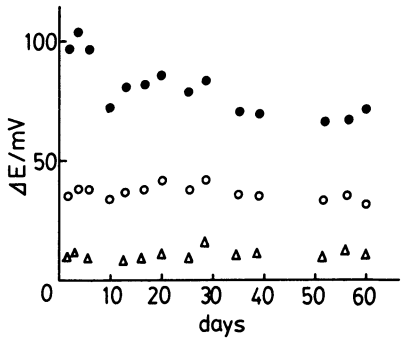


Fig. 9. The long-term stability of the penicillin sensor. The ΔE values were recorded for 10 (—●—), 3 (—○—), and 1 mM (—Δ—) penicillin G in 5 mM phosphate buffer at pH 7.0.

concentration in the solutions from which penicillinase was adsorbed to the electrode. The pH values of the solutions were kept constant at 6.7 by 1 mM phosphate buffer. The penicillin sensor prepared in 0.3% penicillinase solution exhibited the highest response. Unexpectedly, the sensor prepared in 1.3% penicillinase solution showed a decreased response, which may result from the enhanced ionic strength in the 1.3% penicillinase solution. From the results shown in Figs. 6, 7, and 8, it is recommended that, to construct high-performance penicillin sensor, the PVC-COOH-TDA-coated electrode is treated with 0.3% penicillinase solution prepared in 1 mM phosphate buffer at pH 6.7.

The long-term stability of the sensor was examined. The ΔE values of the sensor for 0.1, 3, and 10 mM penicillin G solutions were plotted in Fig. 9. Though the response of the sensor for 10 mM penicillin G solution slightly reduced after 10 days, the sensor can be used for more than 2 months.

Thus, it has been demonstrated that PVC-COOH membrane can be used for immobilizing penicillinase on its surface, as well as for the polymer matrix for making a pH-sensitive base electrode.

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