Voltammetric Response of a γ -Irradiated Poly(*N*-vinylcaprolactam) Modified Graphite Electrode to Catechol and Ascorbic Acid

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Highly discriminative properties between catecholamines and ascorbic acid are desirable for the development of a catecholamine sensor based on electrochemical detection, such as an amperometric or voltammetric type, because of a overlapping of the oxidation potential of catecholamines with that of ascorbic acid. The reason for this is that ascorbic acid is the most serious interferant in the electrochemical detection of catecholamines. In such cases, an electrochemical pretreatment or modification of the electrode has been shown to be effective to improve the selectivity of the sensor against ascorbic acid. A modification of the electrode with an anionic polymer film, such as Nafion^{1,2}, Nafion/polyester ionomer³, styrene/maleic acid copolymer⁴, or negatively charged monolayers⁵, which exclude the penetration of ascorbic acid to the electrode surface, prevents interference from ascorbic acid.

Heineman *et al.* reported on a model catechol sensor based on a poly(*N*-vinylpyrrolidone) (PNVP)-modified electrode⁶⁻⁸, which exhibits an enhanced response to catechol, along with selectivity against ascorbic acid. We have shown that the PNVP membrane had a concentration function with respect to catechol, and that the membrane plays a key role in the observed selectivity and enhanced the sensitivity of the sensor.⁹ Based on the above results, we fabricated a catechol sensor based on an ascorbate oxidase immobilized PNVP-modified electrode, in order to improve the selectivity against ascorbic acid, while maintaining an enhanced response to catechol.¹⁰ Poly(*N*-vinylcaprolactam) (PNVC) is the same type of *N*-vinylamide polymers as is PNVP, in which a five-membered ring of PNVP is replaced by a seven-membered ring, as shown in Fig. 1. If an electrode is modified with PNVC instead of PNVP, the resulting electrode would be expected to show a higher enhanced response to catechol, because the hydrophobic interaction of PNVC with catechol is generally thought to be stronger than that of PNVP, judging from the chemical structures of PNVP and PNVC. In this paper, we wish to report on the response

behavior of a PNVC-modified electrode to catechol and ascorbic acid, compared with the PNVP-modified electrode.

Experimental

Chemicals

The *N*-vinylcaprolactam (NVC) monomer was generously donated by BASF. The NVC monomer was distilled under reduced pressure to remove the inhibitor. Catechol and ascorbic acid of analytical grade were obtained from Kishida Kagaku Co., (Japan) and were used as received. A graphite electrode (4.6 mm ϕ ×3.8 cm-long) was obtained from Poco Graphite, Inc. (USA).

Preparation of PNVC-modified electrode

PNVC was prepared by polymerizing the NVC monomer (10 g) in the presence of 2,2'-azobis(isobutyronitrile) (0.02 g) as an initiator in a 20-ml glass ampoule at 65°C for 72 h. The resulting polymer was dissolved in ethanol and the resulting ethanol solution was added dropwise to hexane. The precipitated polymer was purified by repeated reprecipitation.

A graphite electrode was cleaned by sonicating in water and dried in the air. The side of the graphite electrode was coated with poly(methacrylate) film to insulate by spraying the prepolymer. After drying the

Fig. 1 Chemical structures of PNVC and PNVP: PNVC, poly(*N*-vinylcaprolactam); PNVP, poly(*N*-vinylpyrrolidone).

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Fig. 2 Cyclic voltammograms of catechol. (a) Bare graphite electrode; (b) PNVC-modified electrode. Sample solution, 1 mM catechol solution containing 0.3 M KCl and 0.02 M phosphate buffer (pH 6.8); scan rate, 100 mV/s.

film, one end of the surface of the graphite electrode was polished with emery paper to remove the film, and was then washed with water and dried again. A 15 μ l aliquot of an 8%(w/v) methanol solution of PNVC containing 5%(w/v) NVC was placed on one end of the graphite electrode. After standing over night to evaporate the methanol, the electrode was placed in a glass vial, which was purged with N_2 gas. The electrode was then exposed to ^{60}Co γ -radiation at a dosage of about 20 Mrad. A PNVP-modified electrode was also prepared for a comparison with the PNVC-modified electrode, using the same procedures.

Apparatus and procedure

Cyclic voltammetry was conducted using a BAS-100B electrochemical analyzer (Bioanalytical Systems Inc., USA), and cyclic voltammograms were recorded on an X-Y recorder (Fujitsu, FPG-300, Japan). The electrochemical cell consisted of a Pt auxiliary electrode, an Ag/AgCl (3 M NaCl) reference electrode and the polymer-modified electrode as the working electrode. The peak currents were calculated using the software combined with the BAS-100B electrochemical analyzer. The pH and ionic strength of the sample solution of catechol and ascorbic acid were adjusted to pH 6.8 and 0.3, respectively, by using 0.02 M phosphate buffer and 0.3 M KCl. All sample solutions were deoxygenated by passing N_2 gas through the cell for at least 15 min prior to each experiment.

Results and Discussion

Response to catechol

A thin and shiny film was formed on the surface of the graphite electrode in the dry state as the result of the γ -irradiation. When the PNVC-modified electrode was immersed in the solution, the PNVC polymer on the electrode became swollen and was converted to a transparent membrane of about 1 mm in thickness.

Since the PNVC polymer was cross-linked three dimensionally by γ -irradiation, the polymer did not dissolve in the solution. Prior to each voltammetric experiment, the PNVC-modified electrode was immersed into a blank solution (0.3 M KCl solution containing 0.02 M phosphate buffer, pH 6.8) to let the PNVC membrane become swollen.

Figures 2 (a) and (b) show cyclic voltammograms of catechol obtained by using a bare (unmodified) graphite electrode and the PNVC-modified electrode, respectively, where the concentration of the catechol solution was 1 mM at pH 6.8. These voltammograms were recorded after each electrode was immersed in the solution for 60 min. As can be seen, an anodic peak current for catechol was observed at around +400 mV for both the bare electrode and the PNVC-modified electrode. However, the peak current obtained with the PNVC-modified electrode was about 2.1-times larger than that obtained with the bare graphite electrode. The peak current of the polymer-modified electrode is known to be determined mainly by both the concentration and diffusivity of the solute in the polymer membrane. The former is determined by the partition coefficient of the solutes between a sample solution and the polymer membrane. Generally, since the diffusivity of the solute in the polymer membrane is lower than that in the aqueous solution, the peak current obtained with the polymer-modified electrode is lower than that obtained with the unmodified electrode, if the concentration of the solute adjacent to the electrode is the same in spite of with and without a modification of the electrode. The higher peak current obtained with the PNVC-modified electrode indicates that the concentration of catechol adjacent to the electrode is higher than that in the bulk solution. In other words, catechol is enriched in the PNVC membrane from the bulk solution. Therefore, in spite of the lower diffusivity of catechol in the PNVC membrane, the enhanced response of the PNVC-modified electrode to catechol is due to the larger partition property of the PNVC mem-

Fig. 3 Time-course for the anodic peak current obtained with the PNVC-modified electrode for catechol. Sample solution, 1 mM catechol solution containing 0.3 M KCl and 0.02 M phosphate buffer (pH 6.8); scan rate, 100 mV/s. ●, PNVC-modified electrode; ○, current observed for the blank solution without catechol. A dashed line indicates the anodic peak current obtained with the bare graphite electrode.

brane, as has been observed for the PNVP-modified electrode as well.⁹

The time course of the anodic peak current obtained with the PNVC-modified electrode for the catechol solution is shown in Fig. 3. The peak current at time 0 s is plotted at 0 μA , because the peak current was not observed just before the electrode was transferred to the catechol solution from the blank solution. The anodic peak current increased with time, reaching a constant current value (equilibrium current). The response time required for the current to reach 90% of the equilibrium current was about 30 min. This slow response may have been due to the fact that catechol in the solution diffused into the PNVC membrane immobilized on the graphite electrode, and the concentration of catechol in the PNVC membrane reached a constant value at equilibrium. This transient response corresponds to the diffusion process of catechol through the PNVC membrane. After reaching the equilibrium current, the PNVC-modified electrode was transferred to a blank solution (0.3 M KCl solution containing 0.02 M phosphate buffer, pH 6.8) and the anodic peak current was measured at appropriate time intervals. The anodic peak current rapidly began to decrease, and approached the initial current level. The time course of the anodic peak current after transfer of the PNVC-modified electrode to the blank solution was approximately symmetrical to the time course curve shown in Fig. 3. This indicates that the catechol, which was enriched in the PNVC membrane, diffused out from the membrane to the blank solution. This means that the electrode is renewable for repeated measurements of catechol. However, the anodic peak current obtained using the bare electrode was time-independent.

Figure 4 shows the dependence of the anodic peak current obtained with the bare, PNVP- and PNVC-

Fig. 4 Dependence of the anodic peak current on the concentration of catechol solution. ♦, Bare electrode; ▲, PNVP-modified electrode; ●, PNVC-modified electrode; scan rate, 100 mV/s. The peak currents were adopted at 60 min after immersing the electrodes in sample solution.

modified electrodes on the concentration of catechol in solution. In this case, the values for the peak currents at 60 min after immersing the electrodes in the sample solution were adopted. As shown in Fig. 4, the peak current is proportional to the catechol concentration for the three electrodes. The slope of the peak current vs. catechol concentration plot, which serves as an indicator of the sensitivity to catechol, was 71 $\mu\text{A}/\text{mM}$ for the bare electrode, 121 $\mu\text{A}/\text{mM}$ for the PNVP-modified electrode and 151 $\mu\text{A}/\text{mM}$ for the PNVC-modified electrode. Thus, the sensitivity of the PNVP- and PNVC-modified electrodes was 1.7 times and 2.1 times, respectively, larger than that of the bare electrode. It is noteworthy that the sensitivity obtained with the PNVC-modified electrode was higher than with the PNVP-modified electrode. Molyneux and Frank investigated the interaction between PNVP and a series of aromatic compounds in aqueous solution by an equilibrium dialysis method, and concluded that hydrophobic interactions and charge-transfer interactions are factors in the formation of the PNVP complex with aromatic compounds.¹¹ As mentioned above, PNVC has a molecular structure which is similar to that of PNVP, but the number of carbon atoms in the side chain of PNVC is larger than that of PNVP. The higher sensitivity of the PNVC-modified electrode is presumably due to the fact that the hydrophobic interaction between PNVC and catechol is stronger than that between PNVP and catechol. The stronger interaction of PNVC with catechol results in a higher partition property of PNVC to catechol. Thus, the concentration of catechol in the polymer membrane on the surface of the electrode becomes higher and the sensitivity of the PNVC-modified electrode is larger than that of the PNVP-modified electrode.

Fig. 5 Cyclic voltammograms of ascorbic acid. (a) Bare graphite electrode; (b) PNVC-modified electrode. Sample solution, 1 mM ascorbic acid solution containing 0.3 M KCl and 0.02 M phosphate buffer (pH 6.8); scan rate, 100 mV/s.

Response to ascorbic acid

Figures 5 (a) and (b) show cyclic voltammograms for ascorbic acid obtained with the bare and the PNVC-modified electrode, respectively. The sample solution was a 1 mM ascorbic acid solution containing 0.3 M KCl adjusted to pH 6.8 with a 0.02 M phosphate buffer. These voltammograms were conducted after each electrode was immersed in the solution for 60 min. The time course for the anodic peak current obtained with the PNVC-modified electrode for ascorbic acid, which was observed at around +400 mV, was nearly the same as that observed for catechol. That is, the anodic peak current gradually increased with time, and reached the equilibrium current at about 60 min. However, the equilibrium current obtained with the PNVC-modified electrode was lower by 15% than the anodic peak current obtained with the bare electrode, as can be seen from a comparison with Figs. 5 (a) and (b). The suppressed anodic current obtained with the PNVC-modified electrode indicates that the PNVC membrane may act as a barrier for the permeation of ascorbic acid. Namely, the partition property of the PNVC membrane to ascorbic acid may be small, the same as that for the PNVP membrane.⁹ The anodic peak current at equilibrium obtained with the PNVP-modified electrode was almost identical to that obtained with the PNVC-modified electrode. This means that the partition properties and the diffusivity of ascorbic acid is almost the same for the PNVP membrane and the PNVC membrane.

The data given herein demonstrate that the PNVC-modified electrode exhibited an enhanced response to catechol, and a suppressed response to ascorbic acid. Although no drastic change in the enhanced response to catechol for the PNVC-modified electrode was observed, a slightly larger enhanced response was clearly demonstrated, compared with the response of

the PNVP-modified electrode, which was consistent with our expectation from the chemical structure of the two polymers.

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