Enzyme-exchangeable Enzyme Electrodes employing a Thermoshrinking Redox Gel

Tetsu Tatsuma, Ken-ichi Saito and Noboru Oyama

Department of Applied Chemistry, Faculty of Technology, Tokyo University of Agriculture and Technology, Naka-cho, Koganei, Tokyo 184, Japan

Poly(*N*-isopropylacrylamide-*co*-vinylferrocene) gel acts as an electron mediator between an oxidase and electrode and as an enzyme support, and the enzyme-loaded gel can be unloaded and then reloaded with fresh enzyme or a different enzyme.

Redox polymers as electron mediators between redox enzyme and electrodes need to be hydrophilic for good contact between a water-soluble enzyme and a redox polymer. In the case of a hydrophilic redox polymer, however, enzyme and polymer may have to be cross-linked to avoid dissolution.[1] In recent work,[2] we employed a redox polymer, poly(*N*-isopropylacrylamide-*co*-vinylferrocene)[3,4] (PAF), as an electron mediator and as an enzyme support. PAF is water-soluble at temperature below 20 °C and insoluble above 28 °C. Glucose oxidase (GOx) and PAF were well mixed in water at <10 °C, then cast on the SnO_2 electrode surface and dried. Thus obtained GOx/PAF electrode worked as an amperometric glucose sensor even under nitrogen at 35 °C, where the film is shrunken and insoluble in water. Although such a simple and fast preparation method for enzyme electrode is advantageous over the conventional methods, repetitive use of the enzyme electrodes with PAF is difficult because the PAF film is dissolved gradually in water at a temperature below 20 °C.

The PAF film can be saved from dissolution by cross-linking to form a redox gel [PAF gel, Fig. 1(*a*)].[5] Further, the PAF gel shows thermally induced volume phase transition in a liquid phase[5] as well as some other gels;[6–8] it is swollen <22 °C and is shrunken >35 °C. Therefore, a water-soluble enzyme can be introduced into the PAF gel at <22 °C and the gel holds the enzyme in itself at <35 °C. Further, the trapped enzyme is expected to be released by swelling the gel, and the gel can be loaded again with fresh enzyme of even another kind of enzyme. These processes of loading, measurement, unloading and reloading are schematically illustrated in Fig. 1(*b*). Such an 'enzyme-exchangeable' enzyme electrode has not yet been reported to the best of our knowledge. Further, the PAF gel acts not only as an enzyme support but also an electron mediator between a redox enzyme and electrode as shown below, as well as PAF[2] and some other redox polymers.[1]

Tin oxide (9000 Å thick, F-doped) coated glass plates as the base electrode (Nippon Sheet Glass, Japan) were pretreated with sulfuric acid that had been heated by dilution (1 : 1) for 3–5 min followed by treatment with a 10% toluene solution of methoxydimethylvinylsilane to introduce vinyl groups onto the electrode surface. The electrode surface was covered with a Teflon sheet having an indented zone (area: *ca.* 0.8 cm^2, depth: *ca.* 0.1 mm), which defines where the gel is polymerized. Dimethylsulfoxide containing 9.4 mol dm^{-3} *N*-isopropylacrylamide (recrystallized), 0.31 mol dm^{-3} vinylferrocene (unless otherwise noted), 0.094 mol dm^{-3} *N,N'*-methylenebisacrylamide and 0.34 mol dm^{-3} azoisobutyronitrile was introduced to the gap between the electrode and the Teflon sheet under nitrogen. The electrode was left for 7 h at 60 °C and then the Teflon sheet was removed. Thus obtained PAF gel-coated electrode was washed with Me_2SO and cold water. Geometrical electrode area was *ca.* 1 cm^2, though the area covered with the gel was about 0.6–0.8 cm^2.

The PAF gel coated electrode was loaded with an enzyme, GOx (type II, Sigma, USA) or lactate oxidase LOx (Boehringer, Germany), in a quiescent aqueous solution of the enzyme (*ca.* 17 g dm^{-3}) at temperature below 10 °C, where the gel is swollen, for about 1 h. Then, the temperature was raised to 35 °C to shrink the gel. To unload the enzyme from the gel, the electrode was immersed in quiescent buffer at temperature below 10 °C for several hours.

Electrochemical measurements were performed in a 1/15 mol dm^{-3} phosphate-buffered solution (pH 6.4) at 35 °C. The solution had been saturated with nitrogen before measurements of electrochemical responses, and nitrogen was flowed in the gas phase of the electrolytic cell during the measurements. Reference and counter electrodes were Ag/AgCl/NaCl (saturated) and platinum wire, respectively. Since formal potential of the PAF gel is *ca.* +250 mV *vs.* Ag/AgCl, the

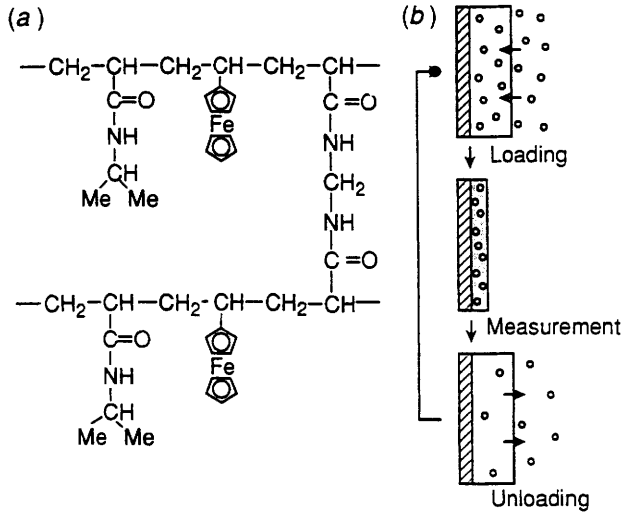

Fig. 1 (*a*) Structure of PAF gel. (*b*) Schematic illustration for the loading, measurement, unloading and reloading processes of the enzyme-exchangeable PAF gel electrode.

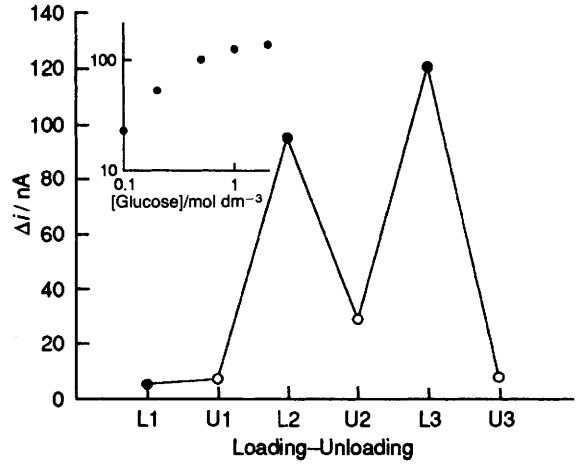

Fig. 2 Steady-state responses of the PAF gel coated electrode, which repeatedly loads with GOx (L1-L3) and unloads (U1-U3), to 1 mmol dm^{-3} glucose in a phosphate buffer (pH 6.4, 35 °C) under nitrogen. Electrode potential is +500 mV *vs.* Ag/AgCl/NaCl (satd.). Inset is a typical calibration curve (second loading).

potential of the enzyme-loaded PAF gel electrode was kept at +500 mV with a potentiostat (LC-4B, BAS) to oxidize the ferrocene sites. Calibration curves were obtained by stepwise injections of standard solutions of a substrate with Eppendorf pipets. Stock solutions of glucose were allowed to equilibrate for mutarotation for at least 24 h before use.

Fig. 2 shows typical steady-state responses of the PAF gel coated electrode, which repeatedly loaded with GOx and unloaded, to 1 mmol dm^{-3} glucose. The responses to glucose indicate that GOx is successfully trapped in the gel. In most cases, the electrode loaded with enzyme for the first time exhibited lower responses than subsequently, as shown in Fig. 2. This may be because it takes a long time to increase affinity of the gel to GOx and to achieve a facile electron transfer. The entrapment of GOx was confirmed by a yellowing section of the bulk gel (*ca.* 10 mm^3), which had been immersed in a GOx solution. Here, we used poly(*N*-isopropylacrylamide) gel (mol ratio of the acrylamides was same as the PAF gel), because the PAF gel is yellow due to vinylferrocene so that entrapment of GOx is difficult to verify.

Inset of Fig. 2 depicts a typical calibration curve obtained for the GOx-loaded PAF gel coated electrode (the second loading). Thus, the GOx-loaded electrode can be used for the glucose determination.

Further, it was revealed that PAF gel can unload GOx. The responses of the unloaded PAF gel electrode were not negligible, but longer immersion in the cold buffer led to smaller responses after unloading.

The responses were obtained even under nitrogen; this indicates that PAF gel functions as an electron mediator between GOx and electrode, because such a response cannot be obtained if electrons abstracted from glucose are not transferred from GOx to the electrode. The reaction scheme is shown in eqns. (1)–(4).

$$\text{GOx} + \text{glucose} \rightarrow \text{GOx(red)} + \text{gluconolactone} \quad (1)$$

$$\text{GOx(red)} + 2\text{Fc}^+ \rightarrow \text{GOx} + 2\text{Fc} + 2\text{H}^+ \quad (2)$$

$$\text{Fc} + \text{Fc}^+ \rightarrow \text{Fc}^+ + \text{Fc} \quad \text{(PAF gel)} \quad (3)$$

$$\text{Fc} \rightarrow \text{Fc}^+ + \text{e}^- \quad \text{(electrode)} \quad (4)$$

where Fc represents the ferrocene site. Electrons are transferred from GOx to the electrode by an electron self-exchange reaction of ferrocene sites, as shown by eqn. (3).

As to the GOx-loaded PAF gel electrode, it takes a relatively long time to obtain a steady-state current in response to glucose injection (*ca.* 30 min). Such a slow response is not practical for sensors. We infer that the slow rate of the electron self-exchange reaction in the PAF gel is responsible for the slow response. Then, we prepared PAF gel from the mixed solution containing twice as much vinylferrocence (0.62 mol dm^{-3}) to increase the concentration of the ferrocene site. In this case, the response time was greatly improved (*ca.* 5–10 min). At present, it is unclear whether the concentrated ferrocene sites or changed film characteristics (*e.g.* enhanced glucose diffusion rate) accelerated the response. It should be noted that Fig. 2 shows data obtained with an original gel (0.31 mol dm^{-3} vinylferrocene).

LOx was also examined as an enzyme for the PAF gel electrode. As a result, the LOx-loaded PAF gel electrode responded to 0.1–1 mmol dm^{-3} L-lactate in a pH 6.4 phosphate buffer (35 °C) at +500 mV. The reaction sequence should be similar to that for the GOx-loaded electrode. Further, it was verified that the electrode can unload LOx and then reload with GOx; the lactate sensor can be changed to a glucose sensor. Thus obtained GOx-reloaded electrode was insensitive to lactate. Similarly, a GOx-loaded electrode was successfully changed to a lactate sensor insensitive to glucose.

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References

1 A. Heller, *J. Phys. Chem.*, 1992, **96**, 3579.
2 T. Tatsuma, K. Saito and N. Oyama, *Anal. Chem.*, 1994, **66**, 1002.
3 M. Irie and T. Tanaka, *Polym. Prepr., Jpn.*, 1991, **40**, 461.
4 N. Oyama, T. Tatsuma and K. Takahashi, *J. Phys. Chem.*, 1993, **97**, 10504.
5 T. Tatsuma, K. Takada, H. Matsui and N. Oyama, unpublished results.
6 T. Tanaka, *Phys. Rev. Lett.*, 1978, **40**, 820.
7 Y. Hirokawa and T. Tanaka, *J. Chem. Phys.*, 1984, **81**, 6379.
8 T. Tanaka, *Sci. Am.*, 1981, **244**, 124.