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A Glucose Biosensor Based on Immobilization of Glucose Oxidase in Electropolymerized *o*-Aminophenol Film on Platinized Glassy Carbon Electrode

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A high-performance amperometric glucose biosensor has been developed, based on immobilization of glucose oxidase in an electrochemically synthesized, nonconducting poly(*o*-aminophenol) film on a platinized glassy carbon electrode. The large microscopic surface area and porous morphology of the platinized glassy carbon electrode result in high enzyme loading, and the enzyme entrapped in the electrodeposited platinum microparticle matrix is stabler than that on a platinum disk electrode surface. The response current of the sensor is 20-fold higher than that of the sensor prepared with a platinum disk electrode of the same geometric area. The experiments showed that the high sensitivity of the sensor is due not only to the large microscopic area but also to the high efficiency of transformation of H_2O_2 generated by enzymatic reaction to current signal on the platinized glassy carbon electrode. The response time of the sensor is <4 s, and its lifetime is >10 months.

Since the pioneering work of Foulds and Lowe,[1] the immobilization of enzymes in an electropolymerized polymer film has proved to be well suited to the preparation of biosensors.[2−20] So far, most of work in this area has been concentrated on use of electropolymerized conducting films of polypyrrole[1−8] and its derivatives,[9,10] and polyaniline.[11,12] However, the recent literature seems to show that much attention has been paid to use of electropolymerized nonconducting polymers such as polyphenol[13,14] and poly(*o*-phenylenediamine).[15−20] The biosensors based on immobilizing enzymes in nonconducting films have some advantages over conducting films: First, the film thickness of the nonconducting polymer is self-controlled during electropolymerization, and a very thin and uniform film can be obtained. The biosensors prepared in this way generally have the advantages of fast response and high sensitivity because of relatively high enzyme loading. Second, the nonconducting polymer films are generally found to be more effective in both preventing the biosensor from fouling and eliminating the interference from electroactive species, such as ascorbic acid and uric acid.

Most of the reported biosensors prepared by the electropolymerization method employ oxidase enzymes as the biocomponent and oxygen as the electron acceptor. The quantitative analysis is based on determination of H_2O_2 liberated by the enzymatic reaction. In most cases, the detection of H_2O_2 is done by measuring its oxidation current on platinum (disk, wire, or plate) electrodes, although the use of other electrode materials has also been reported.[2,20] Since the platinized carbon electrodes possess a number of advantages (e.g., larger surface area and higher catalytic activity) over conventional platinum electrodes, they have received considerable attention and have been used for many analytical purposes.[21−24] They have also been applied to the fabrication of biosensors, in combination with the chemical cross-linking method[25,26] to increase response. However, preparation of biosensors based on immobilization of enzymes in electropolymerized films on a platinized glassy carbon electrode and detailed study of their characteristics, to our best knowledge, have not been reported.

Development of biosensors with high sensitivity and long-term stability will remain a mainstay to meet needs for routine clinical,

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environmental, or industrial applications. In this paper, a highly sensitive and stable amperometric glucose biosensor is developed, based on immobilization of glucose oxidase (GOx) in an electropolymerized *o*-aminophenol (OAP) nonconducting polymer film on a platinized glassy carbon electrode (PGCE). The porous platinum particle matrix of PGCE provides not only a large microscopic surface area for higher enzyme loading but also a desirable microenvironment to transform the enzymatically produced H_2O_2 more efficiently to electronic signal. The experiments also show that the GOx entrapped in a platinized platinum particle matrix is much stabler than that immobilized on a Pt disk electrode (PDE). Therefore, the lifetime of the biosensor was much longer than that of the PDE-based biosensor. Because of the permselectivity and ion-exchange properties of the poly(*o*-aminophenol) (POAP) film, the interference of some electroactive substances, such as ascorbic acid and uric acid, is significantly reduced. A comparison of the PGCE-based sensor with the PDE-based sensor is also presented.

EXPERIMENTAL SECTION

Reagents. Glucose oxidase (EC 1.1.3.4, Type II, 25 000 units/g, purified from *Aspergillus niger*) was purchased from Sigma Chemical Co. *o*-Aminophenol and glucose were obtained from Shanghai Chemical Co. OAP was purified by recrystallization before use. Stock glucose solutions were allowed to mutarotate at room temperature for 24 h before use. All of the other chemicals were analytical grade and were used without further purification. Nitrogen used to obtain a controlled atmosphere in the electrochemical cell was of high-purity grade. All solutions were prepared with doubly distilled water.

Apparatus. All measurements were done using a conventional three-electrode system consisting of a platinum wire counter electrode, a saturated calomel reference electrode (SCE), and a PGCE (3 mm diameter) working electrode (a PDE was also used for comparison). Amperometric measurements and electropolymerization of OAP were carried out on an AD-3 polarographic analyzer (Jintan, China) coupled to an $X-Y$ recorder (Shanghai, China). A magnetic stirrer and bar provide the convective transport. Scanning electron micrograms were obtained with Hitachi S-520 scanning electron microscopy at an accelerating voltage of 20 kV.

Preparation of Enzyme Electrode. Prior to electropolymerization of OAP, the glassy carbon electrode was polished first with diamond paper, followed by 0.5 μm alumina paste, and then sonicated in distilled water to remove any residual alumina. The platinization of glassy carbon electrodes was performed in 0.1 M HCl solution containing 2 mM $PtCl_6^{2-}$ by controlling the potential at -0.3 V vs SCE for 10 min (a typical amount of platinum deposited was $\sim 35-40$ μg). The electrode was then removed from the plating cell and washed thoroughly with 0.05 M, pH 7.0 phosphate buffer. A visible blackish platinum particle film was formed on the glassy carbon electrode surface. Electropolymerization of OAP and immobilization of GOx were performed in an unstirred deaerated 0.05 M acetate buffer (pH 5.6) containing 0.05 M OAP and 20 mg/mL GOx (500 units/mL) by scanning between potentials of -0.2 and $+1.0$ V at a rate of 100 mV/s for 10 min (the charge passing for deposition of POAP/GOx was estimated from the anodic peak area to be 5.7−6.0 mC, and the GOx incorporated was about 0.7 unit). The enzyme electrodes were thoroughly rinsed with doubly distilled water and equilibrated in stirred pH 7.0 phosphate buffer solution for at least 5 min to allow background current to diminish to a steady value. When not in use, the enzyme electrodes were stored in pH 7.0 phosphate buffer at 4 °C.

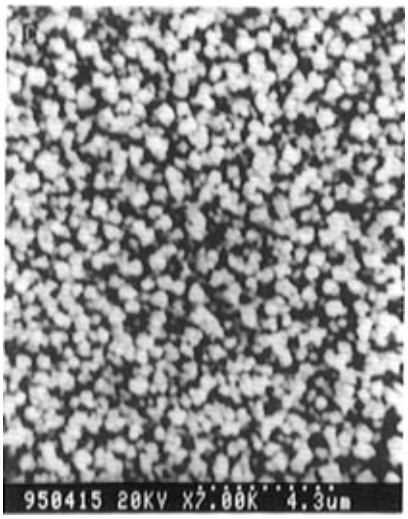

Figure 1. Scanning electron micrograph of the PGCE/POAP/GOx electrode. Magnification: 7000×.

Measurement of the Enzyme Electrode Performance. Amperometric response of the enzyme electrodes to glucose was measured in a stirred, air-saturated 0.05 M phosphate buffer (pH 7.0) by applying a potential of $+0.6$ V to the enzyme electrodes. The background current was allowed to decay to a steady value before aliquots of stock glucose solution were added and the oxidation current of hydrogen peroxide was measured. Unless otherwise indicated, the temperature was kept at 25 ± 0.2 °C during the measurements.

RESULTS AND DISCUSSION

Morphologies of PGCE and the Enzyme Electrode. The morphologies of PGCE- and PGCE-based enzyme electrodes were characterized by scanning electron microscopy (SEM). The SEM of PGCE (not shown) displays a three-dimensional porous open structure. The aggregates of the electrodeposited platinum on the glassy carbon surface are fairly uniform and roughly spherical, with diameters of 0.2−0.5 μm. The porous structure provides a greatly enhanced effective electrode surface for high enzyme loading. When PGCE is coated with electropolymerized POAP or POAP/GOx film (Figure 1), the porous open structure of PGCE is still maintained, although the aggregates get bigger and its surface becomes smoother because of the cover of the film. There is no obvious difference between the SEM of PGCE/POAP and that of PGCE/POAP/GOx.

Electrochemical Polymerization of OAP. Electrochemical polymerization of OAP has been studied extensively in recent years.[27−31] OAP can be electropolymerized in aqueous solution over the entire pH range. The POAP film formed is electroconductive in acidic media, while it is nonelectroconductive in neutral

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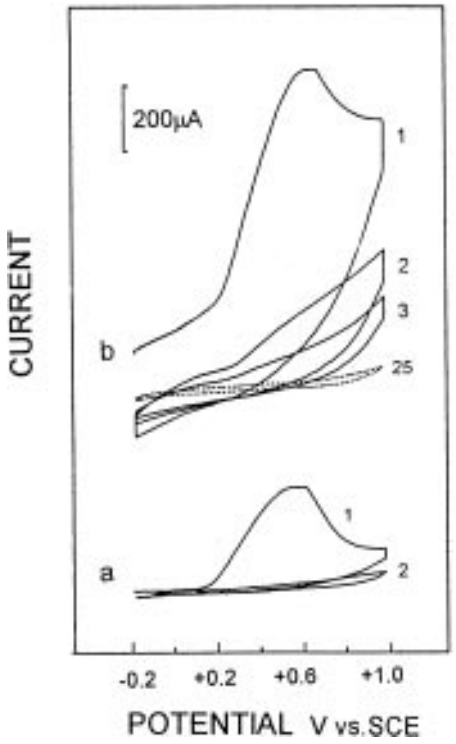

Figure 2. Cyclic voltammograms of electropolymerization of OAP on PDE (a) and PGCE (b). Electropolymerization solution is deaerated 0.05 M acetate buffer (pH 5.6)−0.05 M OAP, with potential scan range from −0.2 to +1.0 V (vs SCE) at a scan rate of 100 mV/s. The numbers on the voltammograms refer to the first, second, third, and 25th cycles, respectively.

and basic solutions.[27,28] The electropolymerization of OAP is different from that of aniline and other aniline derivatives, owing to the easy generation of radical cations from the oxidation of the monomer. The growth of POAP proceeds mainly through the reactions between the polymer and oxidized monomer molecules. The polymer matrix of POAP consists of both oxidized (quinonoid) and reduced (*N*-phenyl-*p*-phenylenediamine) repeating monomer units.[29] The oxidized form of POAP is extremely stable and remains unchanged even after storage in air for 6 months.[27,31]

To avoid denaturation of the enzyme, polymerization of OAP and immobilization of enzyme described in this paper were performed in slight acidic and neutral media, and a nonconducting polymer was formed. Figure 2 shows the typical cyclic voltammograms for the oxidation of OAP at PDE and PGCE in acetate buffer (pH 5.6) solution. The oxidation of OAP is completely irreversible. A broad anodic peak appears in the potential range from +0.2 to +0.8 V on the first scan, and on the second and subsequent scans, the peak current drops significantly with each scan until it finally reaches a minimum value. This indicates that a compact and insulating film is formed which blocks the access of the monomer to the electrode surface.[15] This is probably due to the facts that OAP diffusion in the platinum particle matrix is limited and that the POAP film formed during the scanning process cannot completely cover the electrode surface as quickly as it does on PDE. As a result, the anodic current on PGCE decays more slowly than that on PDE. According to the anodic peak area of the cyclic voltammograms, the amount of charge passing for deposition of POAP on PDE and PGCE is estimated to be ∼1.4−1.6 and 5.7−6.0 mC, respectively. Since the film thickness of nonconducting polymer is self-controlled during the

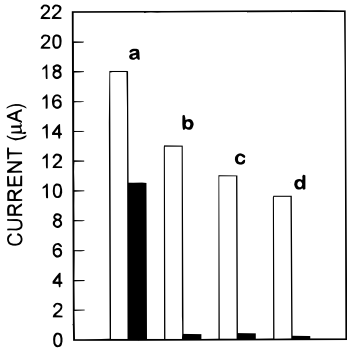

Figure 3. Response of bare PGCE (□) and POAP-coated PGCE (■) to some electroactive substances in phosphate buffer (pH 7.0): (a) H_2O_2, (b) ascorbic acid, (c) uric acid, and (d) $Fe(CN)_6^{4-}$. The concentrations of all the above are 0.1 mM, and the applied potential is +0.6 V.

electropolymerization, the thickness of the POAP film formed on PDE and PGCE should be equal. Thus, the different amount of the charge reflects the difference between the effective surface areas of these two kinds of electrodes.

The POAP film formed under the present conditions is permselective and very useful in both preventing the electroactive species from reaching the electrode surface and keeping the biosensor from fouling. Figure 3 exhibits the responses of some electroactive ions and molecules on a bare PGCE and a POAP-coated PGCE (or a PGCE/POAP/GOx electrode). It shows that the POAP film can reject the access of ascorbic acid, uric acid, and $Fe(CN)_6^{4-}$ to the platinum surface, while it allows ∼60% of H_2O_2 to penetrate it.

Immobilization of GOx. The enzyme electrodes were fabricated by electrochemical polymerization of OAP in the presence of GOx onto the PGCE surface. GOx can be readily incorporated into POAP film during the growth of the polymer. The presence of GOx has little effect on the formation of POAP films (judged from the cyclic voltammograms, not shown). The polymerization of OAP and immobilization of GOx were done either by holding them at a constant potential or by performing cyclic voltammetry. The experiments displayed that the biosensors prepared in the latter way are more reproducible and more effective in rejection of interferents. This must be attributed to the fact that the film formed by cyclic voltammetry is more uniform and compact than that formed at a constant potential.[28] Another advantage of using cyclic voltammetry is that it can follow the film formation on the electrode during electropolymerization.

The various experimental parameters in the GOx immobilization, including the pH of the polymerization solution, the OAP and GOx concentrations, polymerization potential, and deposition time, were explored for optimum analytical performance. The ranges tested for these variables and the optimum conditions are summarized in Table 1. The optimization was based on a comprehensive survey of the response of the sensor to glucose, its stability, and its efficient rejection of electroactive species. The polymerization potential and polymerization time are the most important factors among the variables. It is known that the potential scan range for polymerization can affect the film morphology.[28] Consequently, it can affect the performance of the biosensor. The potential scanning range was selected by keeping a lower switch potential at −0.2 V while changing the upper potential from +0.6 to +1.6 V, and by keeping an upper switch potential at +1.0 V while changing the lower potential from −0.5

Table 1. Optimization of the GOx Immobilization Conditions

experimental variable	testing range	selected value
pH of polymerization solution	3−10	5.6
OAP concentration (M)	0.001−0.1	0.05
GOx concentration (mg/mL)	2−40	20
potential scanning range (V)	−0.5 to +1.6	−0.2 to +1.0
deposition time (min)	1−20	10

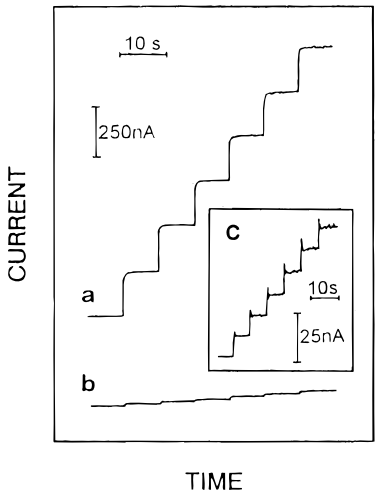

Figure 4. Response characteristics of the biosensor prepared with PGCE (a) and PDE (b, c) to glucose. Curves b and c represent the same response of PDE obtained by adjusting the sensitivity of the apparatus. Successive injection of glucose is on each step of 0.05 mM.

to +0.3 V. As a result, an optimum potential range from −0.2 to +1.0 V was chosen. When the deposition time was varied from 2 to 10 min, the response of the biosensor to glucose only slightly increased, whereas its response to ascorbic acid and uric acid dropped quickly with an increase of deposition time and reached the minimum at 10 min. Further increasing the deposition time led to a slight decrease in response to glucose.

Characteristics of the Biosensors. A typical response of the PGCE/POAP/GOx electrode is demonstrated in Figure 4. The response current of 0.05 mM glucose on the PGCE/POAP/GOx electrode is about 20 times higher than that on the PDE/POAP/GOx electrode. The linear response of the enzyme electrode to glucose is from 1×10^{-6} to 1×10^{-3} M (Figure 5, ●) with response time less than 4 s. The detection limit is 5×10^{-7} M at a signal-to-noise ratio of 3. According to the Lineweaver−Burke form of the Michaelis−Menten equation,[1] the apparent Michaelis−Menten constant (K'_m) for the entrapped enzyme was calculated to be 16.4 mM.

The PGCE/POAP/GOx electrode exhibited very good long-term stability. The stability of the PGCE/POAP/GOx electrode was evaluated by repetitive (200 times) measurements of its response to 1 mM glucose within a period of 10 h. The sensitivity of the sensor lost only 14.3% after the 200 measurements. With intermittent usage and storage at 4 °C in phosphate buffer for 10 months, the biosensor maintained 50% of its original sensitivity and still displayed an excellent response to glucose. A calibration curve of the sensor after 10 months of storage is shown in Figure 5 (○).

The interference from electroactive species, such as ascorbic acid and uric acid, which are generally encountered in determination of physiological sample, is significantly reduced by the use of POAP film (as shown in Figure 3). The presence of 0.1 mM ascorbic acid and uric acid in the buffer containing 1 mM glucose does not affect the response current. A higher concentration of these substances brings about a slightly higher response. However, its response to ascorbate gradually increased when the sensor was stored more than 3 months. The current response to 0.1 mM ascorbate on the sensor stored for 10 months is about 5-fold that on the freshly prepared sensor. This may result from the falling off or dissolution of the film into the solution. Nevertheless, the rejection efficiency can be easily restored by redeposition of OAP onto the electrode surface in a GOx-free OAP solution for 2 min, despite a loss of 30% of its response to glucose.

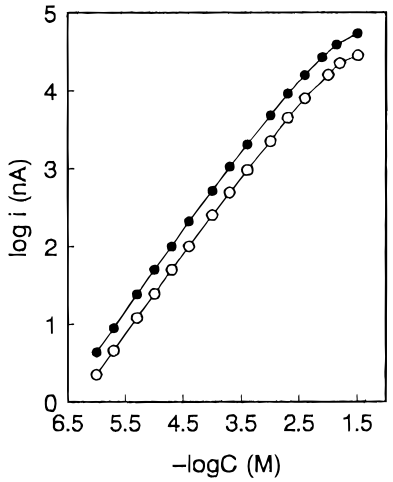

Figure 5. Calibration curves of PGCE/POAP/GOx response to glucose with freshly prepared electrodes (●) and those after 10 months of storage (○).

Investigation of the Mechanism of GOx Incorporation and the Stability of the Biosensors. The mechanism of incorporation of enzymes into electropolymerized polymer film is of great interest and is not very clear yet. We employed several approaches to fabricate GOx biosensors and compared their sensitivity and stability. The results in Figure 6 showed that GOx can be readily adsorbed either on a PGCE surface or on a POAP film surface. These approaches included (a) adsorption of GOx on PGCE by immersing a PGCE in pH 7.0 phosphate buffer containing 20 mg/mL GOx for 10 min; (b) adsorption of GOx on POAP film by immersing a POAP-coated PGCE in the GOx solution described above for 10 min; (c) application of a POAP coating to GOx-adsorbed PGCE after step a; and (d) electropolymerization of OAP and immobilization of GOx simultaneously on PGCE, as described in the Experimental Section.

Figure 6 indicates that all the sensors made with the methods described above exhibited a response to glucose, but their sensitivity and stability differ considerably from sensor to sensor. Both the platinized platinum microparticle matrix surface and the POAP film surface can adsorb GOx. However, the stability of the sensors prepared this way is not very good, since their response was lost almost completely in 3 weeks. The instability of sensors prepared by methods a and b is undoubtedly caused by the gradual desorption of GOx to solution, as the GOx activity was found in the solution which was used to store the sensors. The stability of GOx adsorbed on PGCE can be greatly improved when a POAP film is applied to it, although about half of its response current is reduced. However, it could keep the response

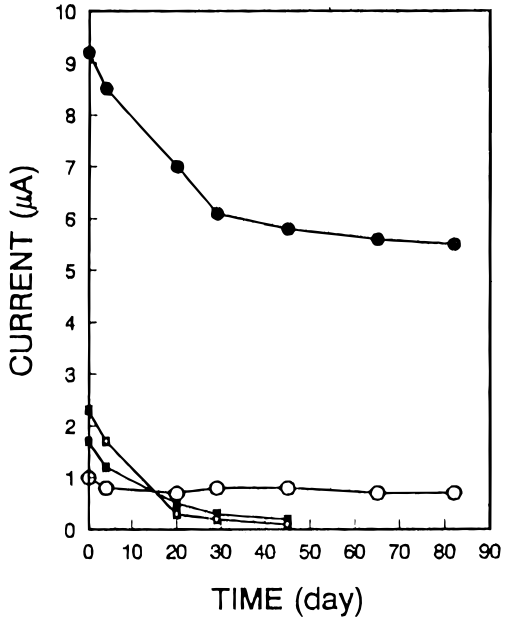

Figure 6. Response current and time course stability of glucose biosensors prepared in different ways: GOx adsorbed on PGCE (■); GOx adsorbed on POAP coated PGCE (□); a POAP film applied on GOx adsorbed PGCE (○); and GOx immobilized simultaneously with the electropolymerization of OAP (●) in the buffer containing 2 mM glucose.

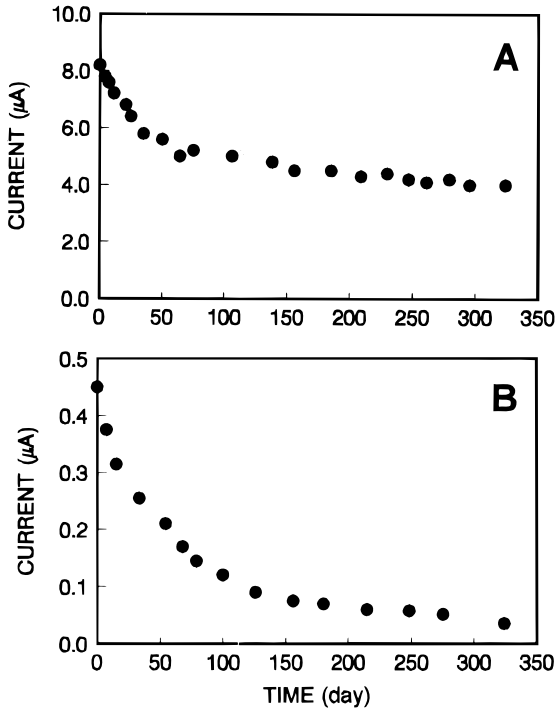

Figure 7. Comparison of the long-term stabilities of the sensors prepared with PGCE (A) and PDE (B) in the buffer containing 2 mM glucose.

almost unchanged within a period of 3 months. This indicates that the POAP film can prevent GOx from leaching and can also partly reject the access of glucose to immobilized GOx. The sensor prepared by approach d is the most sensitive and stablest one. Its response current is about 5-fold higher than that of the sensors prepared with methods a and b, and almost 10-fold higher than that of the sensor prepared with method c. Its response to glucose decreases sharply within the initial 30 days and then reaches an almost steady value. By comparing the stability of the sensors prepared by approach d with b, we can conclude that the quick loss of sensitivity of the GCE/POAP/GOx electrode in the initial 30 days results primarily from the desorption of the GOx adsorbed onto the POAP film surface and that the GOx entrapped within the POAP film is very stable under the storage conditions.

A comparison of the long-term stability of the PGCE-based sensor with that of the PDE-based sensor is shown in Figure 7. The response current of the PGCE/POAP/GOx electrode reached nearly a steady value after it lost 30% of its sensitivity within the initial 30 days. It could keep ~50% of its response after 10 months. In contrast, the PDE/POAP/GOx electrode lost its sensitivity much more quickly; the relatively steady response was reached only after the response was reduced to 20% of its original value. The difference between these two kinds of enzyme electrodes may lie in that desorption and deactivation of the GOx entrapped in the micropores of the platinized platinum particle matrix are not as easy as for those immobilized on the protruding surface or the smooth surface (as PDE surface). The platinized platinum particle matrix provides a favorable microenvironment for stable immobilization of enzymes.

Studies on the Sensitivity. As mentioned above, the response current of the PGCE/POAP/GOx electrode is about 20-fold higher than that of the PDE/POAP/GOx electrode. However, the response to some electroactive species, such as H_2O_2, Fe(CN)$_6^{4-}$, and ascorbic acid, on bare PGCE yields only about 4-fold signal enhancements over that on bare PDE. This is similar to the case reported by Wang and Chen,[20] who utilized an ultrathin porous carbon film to fabricate a glucose sensor by immobilizing GOx in an electropolymerized poly(*o*-phenylenediamine) film. They attributed the enhanced response of the porous carbon film-based sensor to the large microscopic area (vs that of dense film) and the large void volume of the porous film, which offers higher enzyme loading.

An attempt to estimate the activity of the enzyme entrapped in POAP films was made using a method similar to that described by Malitesta et al:[15] A bare PDE was held at +0.6 V in stirred phosphate buffer (pH 7.0, 0.05 M) containing 10 mM glucose. When the background current diminished to a steady value, a known amount of GOx solution was injected or an enzyme electrode was inserted into the solution, and the trace curve (current−time) for the oxidation of H_2O_2 was recorded. The H_2O_2 generation rate was calculated from the trace curve, and a calibration curve was obtained by plotting the initial rate of H_2O_2 production versus enzyme activity. The amount of enzyme in each enzyme electrode is expressed as equivalent activity in solution. The test of PDE/POAP/GOx electrodes showed that the H_2O_2 generation rate in the solution is linearly proportional to the current response of the sensor. That is to say, according to the authors,[15] it is proportional to the amount of GOx incorporated in the polymer film. To our surprise, the test of PGCE/POAP/GOx electrodes gave unexpected results. Although the response current of the PGCE/POAP/GOx electrode is about 20 times that of the PDE/POAP/GOx electrode, the rate of H_2O_2 liberated to the solution is the same as or even smaller than that of the latter, and there is no apparent linear relationship between the response current and the H_2O_2 liberation rate. This phenomenon encouraged us to make further investigations.

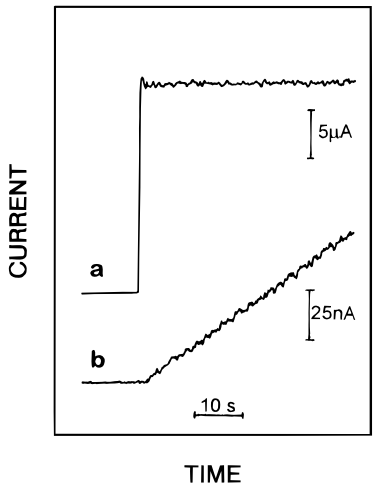

Figure 8. Trace curves for amperometric measurement of glucose on the PGCE/POAP/GOx electrode (a) and for measurement of the H_2O_2 generation rate in the testing solution (b).

We improved the experiment above by using two sets of the three-electrode system in one cell. One set, consisting of a bare Pt disk working electrode, was used to detect the H_2O_2 liberated to solution; the other one, employing the enzyme electrode as working electrode, was utilized for determination of the response current of the sensor. The measurements were carried out in 10 mL of pH 7.0 phosphate buffer at 30 °C. The glucose concentration used was 10 mM. With this improvement, the response curves ($i-t$) (current−time) of the enzyme electrode and the Pt disk electrode for determination of the H_2O_2 generation rate in the solution could be recorded simultaneously (shown in Figure 8). Consider Figure 8 with the following reaction and Faraday's law:

$$H_2O_2 - 2e = O_2 + 2H^+ \quad (1)$$

The rate of H_2O_2 consumed on the enzyme electrode in the amperometric measurement, R_{sen} (μmol/s), and the rate of H_2O_2 diffusing to the solution during the same process, R_{sol}, can be determined. If the H_2O_2 consumed on the Pt disk electrode during the measurement is neglected, the overall H_2O_2 generation rate on the enzyme electrode, R, can be considered as

$$R = R_{sen} + R_{sol} \quad (2)$$

The ratio of H_2O_2 consumed in the amperometric measurement to the total H_2O_2 generated on the enzyme electrode is calculated as

$$\alpha = R_{sen}/R = R_{sen}/(R_{sen} + R_{sol}) \quad (3)$$

We call α the transformation coefficient, which is used to estimate the efficiency of the transformation of H_2O_2 produced by the enzymatic reaction to electronic signal. Therefore, the response current (i) in amperometric measurement can be expressed as

$$i = nF\alpha R \quad (4)$$

where n is the number of electrons transferred and F is the Faraday constant. This means that the response current is dependent on both the H_2O_2 generation rate R and the transformation coefficient α.

The enzyme activity in each enzyme electrode is estimated by using R instead of R_{sol}. Under the optimum polymerization conditions, the typical amount of GOx incorporated in the PDE/POAP/GOx and the PGCE/POAP/GOx is ~170 munits (i.e., 2.4 units/cm^2 of electrode surface) and 700 munits (i.e., 9.9 units/cm^2 of the electrode's geometric surface), respectively.

A determination of α value and related parameters for PGCE/POAP/GOx electrodes and PDE/POAP/GOx electrodes was made, and the results are listed in Table 2.

As shown in Table 2, the total H_2O_2 generation rate on the PGCE/POAP/GOx electrodes is 4-fold higher than that on the PDE/POAP/GOx electrodes. This agrees with the ratio of the charge passing for deposition of POAP/GOx film and the amount of GOx immobilized on the electrode. Furthermore, the transformation coefficient $\alpha = 0.770$ is achieved with PGCE/POAP/GOx electrodes, whereas only $\alpha = 0.153$ is achieved with PDE/POAP/GOx electrodes. The reason for the great difference in α values between these two kinds of electrodes is uncertain; however, the experiments showed that it is closely related to the morphology of the electrode surface. As we know, the H_2O_2 produced by the enzyme reaction in POAP/GOx film or on the electrode surface can diffuse either to the platinum surface, to be oxidized, or out of the film, to be transfered to bulk solution. For the PDE/POAP/GOx electrode, the H_2O_2 diffusing out of the film can be transferred to the stirred test solution immediately by convective transportation, while for the PGCE/POAP/GOx electrode, the H_2O_2 diffusing out of the film stays within the micropore of the platinum particle matrix and is not easily transferred to the outside solution by convective transportation. It is possible that the H_2O_2 diffusing out of the film can repenetrate the film and reach the platinum surface, to be transformed to electronic signal before diffusing out of the matrix into the solution.

From eq 4, the enhanced sensitivity of the PGCE/POAP/GOx electrodes over the PDE/POAP/GOx electrodes can be expressed as

$$i_{PGCE}/i_{PDE} = (\alpha_{PGCE}/\alpha_{PDE})(R_{PGCE}/R_{PDE}) \quad (5)$$

The enhanced sensitivity can be attributed either to the enlarged effective electrode surface area (the second item on the right side of eq 5), which results in higher enzyme loading, or to the higher H_2O_2 transformation efficiency (the first item on the right side of eq 5). In addition, determination of the α and R values with the sensor stored for 10 months displayed that the decrease in sensitivity results mainly from the decreasing R value because of the loss of enzyme activity, while the transformation coefficient α remained unchanged.

CONCLUSIONS

The glucose biosensor based on immobilization of GOx on an electrodeposited platinum microparticle matrix by electropolymerization offered excellent characteristics, including high sensitivity, long-term stability, very short response time, and significantly reduced interference. Detailed studies on the stability and sensitivity of the fabricated biosensor were carried out, and the results showed that the high performance of the sensor is mainly due to the porous structure of the platinum microparticle matrix,

Table 2. Results of Determination of H_2O_2 Generation Rate and Transformation Coefficient α

	Q (mC)	GOx (munits)	i (μA)	R_{sen} (μmol/s)	R_{sol} (μmol/s)	R (μmol/s)	α
PGCE/POAP/GOx (a)	5.8	700	28.75	1.49×10^{-4}	4.44×10^{-5}	1.93×10^{-4}	0.770
PDE/POAP/GOx (b)	1.5	170	1.40	7.26×10^{-6}	4.00×10^{-5}	4.73×10^{-5}	0.153
ratio of (a) to (b)	3.9	4.1	20.5	20.5	1.1	4.1	5.0

which provides a favorable microenvironment for GOx loading. GOx immobilized in the micropores of platinum particle matrix is not easy to desorb or leach to solution and may be also not easy to deactivate. The greatly enhanced sensitivity is due not only to the high enzyme loading but also to the high efficiency of transformation of enzymatically generated H_2O_2 to electrical signal. These results provide a guide for design and fabrication of the highly sensitive biosensors. To construct a sensitive biosensor, the high enzyme loading is not the only factor that should be considered: the high substrate−signal transformation efficiency is also a significant factor that should be taken into account. However, little attention has been paid to the latter until now. In addition, the characteristics of the platinized surface have great promise for the development of highly sensitive miniature biosensors. The film material, the film morphology, and the electropolymerization conditions also can affect the H_2O_2 transformation efficiency besides the morphology of the electrode surface. Studies on these aspects are being carried out in our laboratory.

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