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Short communication

Electropolymerization of preoxidized catecholamines on Prussian blue matrix to immobilize glucose oxidase for sensitive amperometric biosensing

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ABSTRACT

We examine here the electropolymerization of electrochemically or chemically preoxidized catecholamines in glucose oxidase (GOx)-containing neutral solutions to efficiently immobilize the enzyme at Prussian blue-modified Au electrodes for sensitive amperometric biosensing of glucose. The electrochemical quartz crystal microbalance (EQCM) was used to track various electrode-modification processes. The optimized poly(dopamine)-based glucose biosensor displayed a sensitivity of 35 μA mM^{-1} cm^{-2} and a limit of detection of 0.3 μM at 0.7 V vs. SCE, and similar results were obtained at −0.05 V vs. SCE, which are obviously better than those from preoxidation-free conventional electropolymerization. The immobilized GOx retained high enzymatic specific activity, as quantified by UV–vis spectrophotometry and EQCM. L-Noradrenalin could similarly electropolymerize and the resultant enzyme film gave equivalent biosensing characteristics, but the electropolymerization of EP was less efficient and the resultant enzyme film showed notably poorer performance.

1. Introduction

Catecholamines (CAs), including dopamine (DA), noradrenaline (NA) and epinephrine (EP) that are structurally depicted in Scheme 1S in the Supplemental Data, are important hormones and neurotransmitters that have been attracting wide attention (Corona-Avendaño et al., 2005; Elwing et al., 1990; Hawley et al., 1967; Levis et al., in press; Ling et al., 2005; Schwarz and Hauser, 2003; Walaas, 1963). Due to their intrinsic redox nature, many electrochemical studies on DA, NA and EP have been conducted (Chen and Peng, 2003; Hawley et al., 1967; Lisdat et al., 1997; Luczak, 2008). Recently, the DA polymer from conventional electropolymerization (DAP_{CEP}) has been developed as an excellent matrix to immobilize antihuman immunoglobulin G, glucose oxidase (GOx) and hemoglobin (He et al., 2005; M.R. Li et al., 2006a,b). Also, the self-polymerization of DA was proposed for multifunctional thin coatings on a wide range of inorganic and organic materials (Lee et al., 2007). However, as far as we know, the possible electropolymerization of NA and EP, including their biomolecules-immobilization applications, has been paid insufficient attention to date.

The high-load and high-activity immobilization of enzyme is a big concern in the biocatalysis and biosensing fields (Karyakin et al., 2002). Very recently, we reported the integration of chemical and electrochemical polymerization for high performance biosensing (Fu et al., 2008). However, the electropolymerization of preoxidized CAs has not been screened and examined to immobilize enzymes for sensitive amperometric biosensing.

Herein, we examine into the electropolymerization of electrochemically or chemically preoxidized CAs in GOx-containing neutral aqueous solution to immobilize GOx on Prussian blue-modified Au electrode (PB/Au) for sensitive amperometric biosensing of glucose. The enzyme electrodes based on DA and NA polymers involving preoxidization (DAP_{PO} and NAP_{PO}) as enzyme-immobilization matrices exhibit good performance, but the electropolymerization of EP is less efficient for biosensing application. The PB matrix enables us to sensitively determine glucose in both H_2O_2-oxidation and H_2O_2-reduction modes (Lin et al., 2004; Ricci and Palleschi, 2005; Zhang et al., 2004). The quantity and enzymatic specific activity (ESA) of the immobilized enzyme are evaluated through electrochemical quartz crystal microbalance (EQCM) and UV–vis spectrophotometry.

2. Experimental

2.1. Instrumentation and reagents

The instrument and reagents are given in the Supplemental Data, with most of them identical to our earlier report (Fu et al., 2008).

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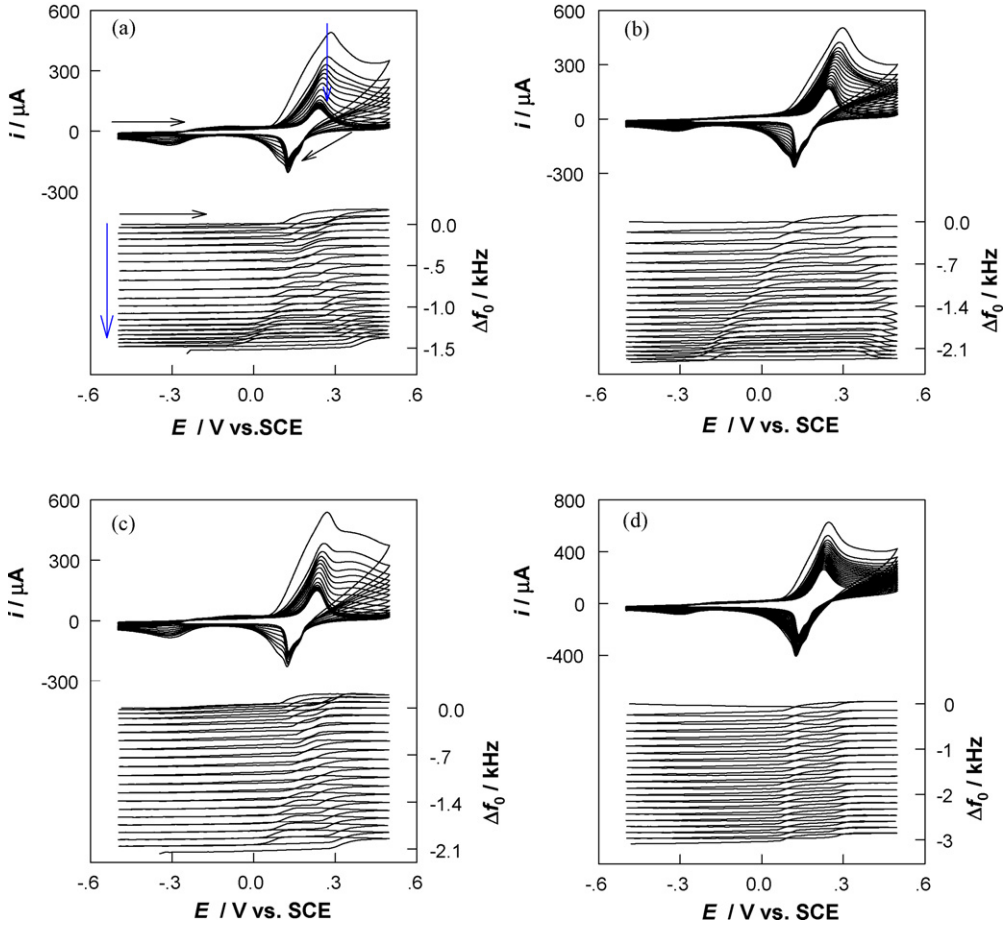

Fig. 1. Simultaneous current (i) and Δf_0 responses at −1.5 kHz PB/Au to potential cycling in 30 mM aqueous PBS (pH 7.0) containing 30 mM DA (a), 30 mM DA + 3.0 mM $K_3Fe(CN)_6$ (b), 30 mM DA + 3.5 mg mL^{-1} GOx (c), or 30 mM DA + 3.5 mg mL^{-1} GOx + 3.0 mM $K_3Fe(CN)_6$ (d). Scan rate: 20 mV s^{-1}.

2.2. Procedures

The procedures for GOx immobilization and amperometric biosensing are illustrated in Scheme 2S. A PB layer (−1.5 kHz after optimization) was electrodeposited on Au. For conventional electropolymerization (CEP), the one-step electrochemical codeposition of CA polymer (CAP) and GOx in an aqueous solution containing CA monomer and GOx yielded an enzyme film on PB/Au. In the preoxidation case, the solution of CA plus GOx was firstly subject to preoxidation through the application of chemical preoxidants (solution unstirred) or an anodic potential (0.20 V, solution stirred) for 1 h, yielding many CAP–GOx composites, some of which were then codeposited during electrochemical polymerization of CA on PB/Au. The enzyme electrodes were thoroughly washed with pure water before use, and then we did not observe any current from the probably entrapped preoxidant. The electropolymerization was conducted via cyclic voltammetry (CV) under solution-unstirred conditions. The fresh prepared enzyme electrode was water-rinsed and stored in phosphate buffer solution (PBS, pH 7.0) at 4 °C when not in use.

3. Results and discussion

3.1. Electropolymerization of DA, NA or EP on Au

Fig. 1S in the Supplemental Data shows the EQCM response on bare Au during potential cycling in neutral PBS containing 5.0 mM DA, NA or EP. A DA-oxidation current peak was observed at ca. 0.20 V in the first positive scan, two cathodic peaks roughly at 0.06 and −0.32 V were recorded in the negative scan, and a new oxidation peak (ca. −0.25 V) was obtained in the second positive scan, which can be well explained by the well-known ECE mechanism of DA electrochemistry (Chen and Peng, 2003; Y.L. Li et al., 2006). The frequency decrease was observed at potentials positive of ca. 0.06 V in each cycle, indicating DA electropolymerization and the polymer deposition on the electrode. NA exhibited EQCM responses similar to DA, but EP polymerized to a smaller degree, with slower decreases in current peaks and frequency. According to our previous study on DA electropolymerization (Y.L. Li et al., 2006), the preliminary mechanisms for the polymerization of CAs are briefly described in Scheme 1S, namely, CA is electrooxidized to catecholaminequinone (CAQ, E_{pa} = 0.20 V); the intramolecular cyclization reaction of CAQ via 1,4-Michael addition leads to the more readily oxidizable leucocatecholaminechrome (LCAC); then the LCAC is oxidized to catecholaminechrome (CAC, E_{pa} = −0.25 V). The CAC can further undergo indole-like polymerization on the electrode, yielding deposited melanin-like polymers. We found that the CV behaviors of all the CAs-electropolymerized films were very similar to that of polyindole in 1.0 mol L^{-1} aqueous $HClO_4$ (Saraji and Bagheri, 1998), and the main infrared peaks of them were also very similar to those for polyindole (not shown), supporting the proposed indole-like electropolymerization of CAs. With the DA case examined in detail, we will mainly study the applications of the CAs-electropolymerized films as biocompatible matrices in developing biosensors.

3.2. Preparation and performance of enzyme electrodes

The electrodeposition of PB and the electrocatalyzed oxidation of H_2O_2 on PB/Au were examined via EQCM. Fig. 2S(a) in the Supplemental Data shows current and Δf_0 responses to the CV deposition of PB in 0.50 mM $K_3Fe(CN)_6$ + 0.50 mM $Fe_2(SO_4)_3$ + 0.10 M K_2SO_4 + 0.05 M H_2SO_4 solution (30 mV s^{-1}, 0.35 to −0.05 V) (Deng et al., 2006). With the progress of potential cycling, the EQCM frequency kept decreasing, and a pair of well-defined current peaks near 0.15 V, characteristic of redox switching between PB and Prussian white (PW), was increasing (Zou et al., 2007). The addition of 5.0 mM H_2O_2 heightened the reduction peak of PB but shortened the oxidation peak of PW, proving the efficient electrocatalyzed reduction of H_2O_2 on PB/Au (Fig. 2S(b)). The PB film thickness and detection potential based on potentiostatic reduction/oxidation of H_2O_2 were optimized in stirred 0.50 mM H_2O_2 solution, yielding an optimized PB film thickness of ca. −1.5 kHz frequency shift (PB load of 8.2 μg cm^{-2}, according to the Sauerbrey equation) and optimized detection potentials at −0.05 and 0.70 V, respectively (Fig. 2S(c)).

Then, the EQCM was used to track the electrodeposition of DAP_{CEP} and DAP_{PO} on PB/Au in the presence and absence of GOx. Fig. 1(a) shows the simultaneous records of current and Δf_0 during 20-cycle potential cycling in PBS (pH 7.0) containing 30 mM DA to develop a DAP_{CEP} film on PB/Au. All current peaks were being shortened with the progress of potential cycling, indicating a gradual decrease in the electrode activity due to the deposition of a non-conducting DA polymer layer. Fig. 1(b) shows the deposition of DAP_{PO} on PB/Au by cycling in PBS containing 30 mM DA + 3.0 mM $K_3Fe(CN)_6$. The final $-\Delta f_0$ was larger than that for DAP_{CEP} by 0.70 kHz (6% R.S.D. in three repeated runs), indicating an enhanced polymer electrodeposition in the preoxidation case under comparable conditions. Fig. 1(c and d) show EQCM responses to potential cycling in PBS containing 30 mM DA + 3.5 mg mL^{-1} GOx (c) and 30 mM DA + 3.5 mg mL^{-1} GOx + 3.0 mM $K_3Fe(CN)_6$ (d), respectively, with other conditions identical to Fig. 1(a and b). In comparison with the GOx-free cases, greater $-\Delta f_0$ by 0.60 (c vs. a) and 0.80 kHz (d vs. b) were observed under comparable polymerization conditions, indicating that the GOx was immobilized in the film and the preoxidation treatment is effective in increasing the amount of immobilized enzyme, as also validated by the subsequent biosensing experiments. The potential cycling number of 20 was found to be optimum and thus fixed in the following detections.

Concentrations of DA, $K_3Fe(CN)_6$ and GOx in the polymerization bath for GOx immobilization were optimized, as shown in Fig. 3S in the Supplemental Data. The optimal conditions at DAP_{PO}–GOx/PB/Au were obtained as 30 mM DA, 3.0 mM $K_3Fe(CN)_6$ and 3.5 mg mL^{-1} GOx. Fig. 4S shows the effects of solution pH and applied glucose-detection potential on the current response of the prepared enzyme electrode, with maximum response observed near pH 7.0, as well as at 0.70 V (H_2O_2-oxidation mode) and at −0.05 V (H_2O_2-reduction mode). These optimal conditions were fixed for the following experiments.

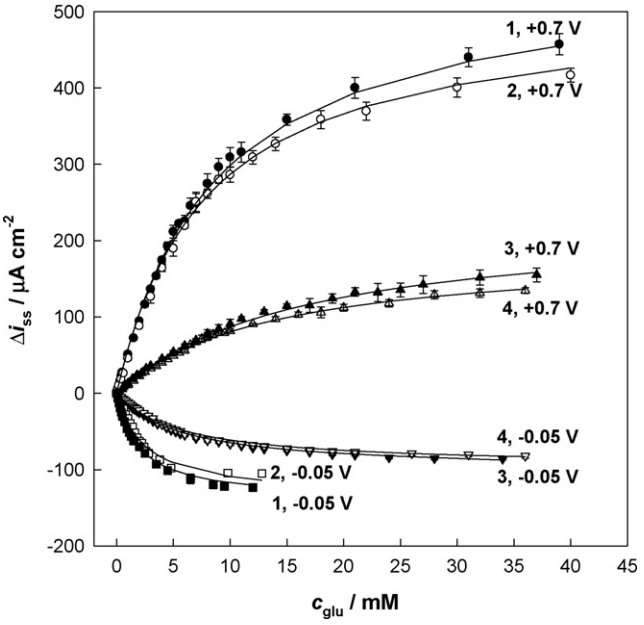

Fig. 2. The calibration curves for DAP_{PO}–GOx/PB/Au (1), NAP_{PO}–GOx/PB/Au (2), DAP_{CEP}–GOx/PB/Au (3) and NAP_{CEP}–GOx/PB/Au (4) at 0.70 and −0.05 V in 30 mM aqueous PBS (pH 7.0). Roughly equivalent RSD (~5%) of the biosensing responses at −0.05 V were also obtained. The curves show the corresponding results for nonlinear fitting to the Michaelis–Menten kinetics (see the Supplemental Data for details).

Fig. 2 shows typical steady-state current responses at DAP_{PO}–GOx/PB/Au, NAP_{PO}–GOx/PB/Au, DAP_{CEP}–GOx/PB/Au, and NAP_{CEP}–GOx/PB/Au (NAP_{CEP} stands for NA polymer from CEP) to successive additions of glucose under optimized experimental conditions, and the main results for DA are summarized in Table 1. The preoxidation treatment in the DA case increased the glucose-detection sensitivity to 35 ± 1.8 μA mM^{-1} cm^{-2} at 0.70 V (ca. 3.3 fold of that from CEP), with the limit of detection (LOD) decreased by an order of magnitude and the apparent Michaelis–Menten constant (K_m^{app}) obviously decreased (Yang et al., 2002). Similar results were recorded at the detection potential of −0.05 V. The response performance was roughly equivalent in the NA case. Good selectivity and stability of

Table 1
Characteristics of DAP_{PO}–GOx/PB/Au and DAP_{CEP}–GOx/PB/Au enzyme electrodes.

Enzyme electrode	DAP_{PO}–GOx/PB/Au		DAP_{CEP}–GOx/PB/Au	
Detection potential (V)	0.70	−0.05	0.70	−0.05
Linear range (mM)	0.005–5.5	0.004–2.4	0.05–9	0.05–3.2
Sensitivity (μA mM^{-1} cm^{-2})	35 ± 1.8	35 ± 1.4	10.5 ± 0.6	10 ± 1.0
K_m^{app} (mM)	8.6 ± 0.2	1.9 ± 0.1	16 ± 0.5	5.7 ± 0.1
LOD (μM)	0.3	0.3	2	2
Enzyme activity[a]				
$-\Delta f_{0,GOx}$/kHz	0.80 ± 0.05		0.60 ± 0.03	
EQCM estimation				
ESA_n/kU g^{-1}	74 ± 4			
ESA_i/kU g^{-1}	34 ± 1		16 ± 0.6	
ERA/%	46 ± 1		22 ± 1	
Spectrophotometric estimation				
ESA_n/kU g^{-1}	79 ± 6			
ESA_i/kU g^{-1}	64 ± 5		33 ± 2	
ERA/%	81 ± 6		42 ± 3	

[a] ESA_i and ESA_n are the ESA for immobilized GOx and native GOx, respectively, and the enzyme relative activity (ERA) is the ratio of ESA_i to ESA_n.

the resultant enzyme electrodes were also obtained, as shown in Figs. 5S and 6S in the Supplemental Data.

3.3. Brief discussion on the sensitivity enhancement of the enzyme electrodes via preoxidation

The electrochemical preoxidation in a stirred solution was also examined here for the first time and an anodic potential (0.20 V) was screened out for CA preoxidation. Several other chemical preoxidants ($K_2Cr_2O_7$, H_2O_2, $Ce(SO_4)_2$, $Fe_2(SO_4)_3$, and *p*-benzoquinone) were also examined (Table 1S). Biosensors fabricated with polymers from DA, NA, and EP were examined and compared in Table 2S. The sensitivity of the biosensors prepared after preoxidation were found to be 2–4-fold improved versus those via CEP. The utilization of $K_3Fe(CN)_6$ as the preoxidant and DA as the monomer gave the best performance.

The enzymatic load and activities of the immobilized GOx were quantitatively estimated via EQCM and UV–vis spectrophotometry (Bergmeyer, 1963; Su et al., 2008), as summarized in Table 1, and the operation details are given in the Supplemental Data. Both techniques demonstrated that the ESA of the immobilized GOx (ESA_i) was enhanced roughly by 2-fold after preoxidation treatment. The somewhat lower ESA_i obtained from EQCM was also found here, due probably to the not complete capture of enzymatically generated H_2O_2 at the electrode (Fu et al., 2008).

The scanning electron microscopy and atomic force microscopy were applied to study the surface morphology of enzyme films, as shown in Fig. 7S. The DAP_{CEP}–GOx/PB/Au surface was relatively smooth, but the DAP_{PO}–GOx/PB/Au surface was obviously studded with many size-enlarged nanoparticles (ca. 0.1 μm), indicating DA oligomer composites of larger size formed during the preoxidation treatment and anchored on the electrode (Ćirić-Marjanović et al., 2007; Stover et al., 2007). Since the enzyme molecules are in higher freedom in solution than at the electrode/solution interface, the solution-state polymerization is favorable to entrap high-activity enzymes, and the subsequent electropolymerization of the remaining CA monomer after the preoxidation to anchor the GOx–polymer composites on the electrode should cause less conformational change of the entrapped enzyme, yielding high-performance enzyme films. However, in the CEP case, the soft enzyme molecules just near the interface are dragged onto the electrode surface during monomer electropolymerization, thus, the enzyme is less immobilized and the immobilized enzyme may be more distorted and deactivated.

4. Conclusions

The electropolymerization of DA, NA and EP has been comparatively examined for the first time, and the polymers of DA and NA, rather than EP polymer, were found to be excellent matrices for GOx immobilization under our experimental conditions. The high-load and high-activity immobilization of GOx has been achieved with the electrodeposited polymer of preoxidized DA or NA as matrices, yielding high-performance enzyme electrodes. The biocompatible polymer matrices from facile polymerization of DA and NA may be extended to many other biosensing and bioelectronic applications.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2008.12.016.

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