Talanta 85 (2011) 43–48

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Bilayer lipid membrane biosensor with enhanced stability for amperometric determination of hydrogen peroxide

Longzhen Zheng*, Leyan Xiong, Dan Zheng, Yindi Li, Qiang Liu, Kui Han, Wen Liu, Kun Tao, Shaoming Yang, Jian Xia

Department of Chemistry and Chemical Engineering, East China Jiao Tong University, Nanchang, Jiangxi 330013, PR China

ARTICLE INFO

Article history:
Received 12 November 2010
Received in revised form 18 February 2011
Accepted 26 February 2011
Available online 4 March 2011

Keywords:
Polydopamine
Electropolymerization
Bilayer lipid membrane
H_2O_2 biosensor
Storage stability

ABSTRACT

In this paper, a polydopamine (PDA) film is electropolymerized on the surface of bilayer lipid membrane (BLM) which is immobilized with horseradish peroxidase (HRP). The coverage of the PDA film on HRP/BLM electrode is monitored by electrochemical impedance spectroscopy (EIS). The electrocatalytic reduction of H_2O_2 at the PDA/HRP/BLM electrode is studied by means of cyclic voltammetry (CV). The biosensor has a fast response to H_2O_2 of less than 5 s and an excellent linear relationship is obtained in the concentration range from 2.5×10^{-7} to 3.1×10^{-3} mol L^{-1}, with a detection limit of 1.0×10^{-7} mol L^{-1} (S/N = 3). The response current of BLM/HRP/PDA biosensor retains 84% of its original response after being stored in 0.1 mol L^{-1} pH 7.0 PBS at 4 °C for 3 weeks. The selectivity, repeatability, and storage stability of PDA/HRP/BLM biosensor are greatly enhanced by the coverage of polydopamine film on BLM.

1. Introduction

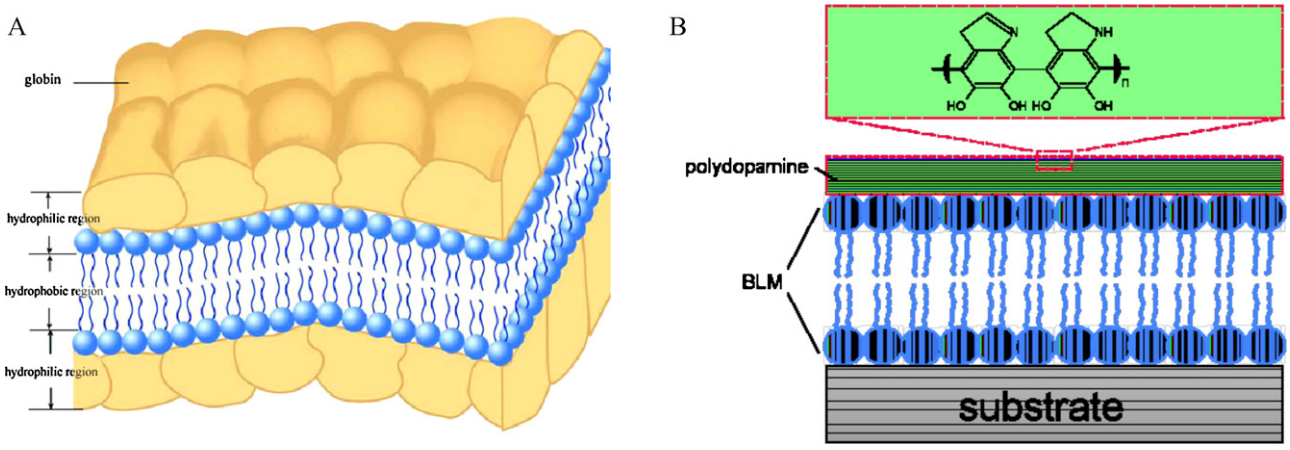
In biological systems, the cellular membranes play a key role in the selective transport of molecules, receptor binding, enzymatic activity, and control of cell–cell interactions. The function of biomembranes is closely related to their structures, which embedded proteins in the lipid membranes [1,2]. Lipids are amphiphilic molecules, which can spontaneously form bilayer structures in aqueous media. Natural cell membranes consist mainly of different lipids such as phospholipids, glycolipids, cholesterol and proteins. Compared with all other immobilization matrices, lipid membranes provide a natural environment for embedding biomolecules, and the biosensors display fast response times and high sensitivities [3]. Furthermore, the presence of the lipid bilayer greatly reduces background noise (interferences) and effectively excludes macromolecules that could block the electron transfer of proteins and hydrophilic electroactive compounds from reaching the detection surface [4].

Since the discovery of planar bilayer lipid membranes by Müller et al. [5], bilayer lipid membranes (BLMs) have been extensively studied as an ideal model membranes that reveal the basic properties of biological membranes. However, the low mechanical and electrical stability of the "free-standing" planar lipid bilayers restricted their practical applications in biosensors. In order to stabilize the lipid films, it is often necessary to immobilize BLMs on solid substrates, such as metal [6], hydrogel [7], Si and polymer [8], and glassy carbon [9]. Much progress has been achieved in stabilizing lipid films in electrochemical biosensors [10]. For example, horseradish peroxidase (HRP) was immobilized in the BLMs supported by the redox polyaniline (PAN) film for the development of H_2O_2 biosensor [11].

The stability of s-BLMs is improved to some extent comparing with that of planar lipid bilayer. However, most of s-BLMs only have a lifetime of several hours or days. So it is still a challenge for the development of BLMs with long-term stability. Our strategy is inspired by the structure of biomembranes. In the structure of biomembranes, BLMs are covered with hydrophilic globin proteins (Scheme 1A). This structure leads to the high stability of biomembranes. It has been reported that polydopamine (PDA) is the main composition of adhesive proteins secreted by mussels for attachment to wet surfaces [12,13]. PDA performs well as binding agents for coating inorganic surfaces [14–20], including the electropolymerization of dopamine onto conducting electrodes [21]. In the present paper, BLM is first self-assembled on the surface of glassy carbon electrode, and then horseradish peroxidase (HRP) is immobilized in the s-BLM for the preparation of H_2O_2 biosensor. Finally, a PDA thin film is electropolymerized on the surface of s-BLM (Scheme 1B). The resulting biosensor shows rapid electrocatalytic response and high sensitivity for monitoring H_2O_2. The stability of PDA/s-BLM is also greatly enhanced comparing to s-BLM.

* Corresponding author. Tel.: +86 791 7046351; fax: +86 791 7046320.
E-mail address: zlz@ecjtu.jx.cn (L. Zheng).

0039-9140/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2011.02.048



Scheme 1. Structure of biomembrane (A) and polydopamine/s-BLM (B).

2. Experimental

2.1. Reagents

Horseradish peroxidase (HRP, EC 1.11.1.7, RZ > 3.0, A > 250 U/mg), dopamine hydrochloride, and dimyristoylphosphatidylcholine (DMPC, 99%, TLC) are purchased from Sigma Chemical. Hydrogen peroxide (30%) is purchased from Beijing Chemical Reagent Co. (Beijing, China). Chloroform is analytical grade and is used without further purification. The phosphate buffer solution (PBS) contains 5 mmol L^{-1} NaH_2PO_4, 5 mmol L^{-1} Na_2HPO_4 and 0.1 mol L^{-1} NaCl. All solutions are made up with twice-distilled water.

2.2. Apparatus

Electrochemical measurements are performed on a CHI 660A electrochemical workstation (Chenhua, China) with a conventional three-electrode system with the enzyme electrode as working electrode, a platinum wire as auxiliary electrode, and an Ag/AgCl (saturated KCl) electrode as reference against which all potentials are measured.

Electrochemical impedance spectra (EIS) measurements are conducted by a Solartron 1255B frequency response analyzer combined with a Solartron 1286 electrochemical interface (Solartron Farnborough, UK). An IEEE-interface (National Instruments, USA) is employed to couple the two Solartron instruments with a computer.

2.3. Preparation of enzyme electrode

Glassy carbon electrode (diameter of 4 mm) are first polished with 1.0, 0.3, and 0.05 μm α-alumina slurry subsequently, and followed by rinsing thoroughly with doubly distilled water. The electrodes are then successively sonicated in 1:1 nitric acid, acetone, and doubly distilled water and then allow to dry at room temperature.

DMPC is dissolved in chloroform to give a final concentration of 2 mg ml^{-1}. A 5 ml aliquot of the lipid solution is dropped onto the surface of the electrode by a microsyringe and the electrode is immediately transferred in the phosphate buffer solution, in which the supported bilayer lipid membrane is formed spontaneously. Then the BLM modified electrode is immersed in 2 mg ml^{-1} HRP solution for 10 h to incorporate HRP into the BLM. Finally, a polydopamine (PDA) film is electropolymerized on the HRP/BLM modified electrode in 0.1 mol L^{-1} PBS solution (pH = 7.0) containing 2.5 mg ml^{-1} dopamine via cyclic voltammetry (30 cycles) with the scan rate of 100 mV s^{-1} and the scan range from −0.2 to 0.6 V. The PDA/HRP/BLM electrode is rinsed thoroughly with doubly distilled water and kept in 0.1 mol L^{-1} PBS at 4 °C in a refrigerator before use.

2.4. Measuring procedure

Cyclic voltammetric measurements are done in an unstirred electrochemical cell. Amperometric experiments are carried out by applying a potential step of −50 or −150 mV to a stirred electrochemical cell. Aliquots of a standard solution of H_2O_2 are successively added to the solution. Current–time data are recorded after a steady-state current have been achieved. All experimental solutions are deaerated by high pure nitrogen for 10 min, and a nitrogen atmosphere is kept over the solutions during measurements.

The impedance spectra are recorded using an ac perturbation signal of 5 mV within the frequency range of 10^6–0.01 Hz. The impedance data are fitted with an equivalent circuit using the *Z* plot/*Z* view software (Scribner Associates Inc., England). The equivalent circuit provides an electrical analogue of the chemical/physical processes probed by EIS. Impedance measurement is performed in the presence of a 10 mmol L^{-1} $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) mixture containing 0.1 mol L^{-1} KCl as a redox probe at the formal potential of the system.

3. Results and discussion

3.1. Electropolymerization of polydopamine

Fig. 1A shows typical cyclic voltammogram for the electropolymerization of polydopamine on bare GCE electrode. Two pairs of peaks at 0.21/0.12 V and −0.20/−0.26 V are observed. The anodic peak at 0.21 V is responsible for dopamine oxidation to ortho-dopaminoquinone and the cathodic peak at 0.12 V is related to ortho-dopaminoquinone transformation to dopamine. The redox pair with the anodic and cathodic peak at −0.20 V and −0.26 V is associated with reversible oxidation of leucodopaminochrome to dopaminochrome [21]. On successive scans, the peak currents decrease cycle-by-cycle. It suggests a compact and insulating film is formed and coated onto the electrode surface progressively, which leads to the reduction of the voltammetric response. The cyclic voltammogram for electropolymerization of polydopamine on HRP/BLM electrode is similar with that on bare GCE electrode (Fig. 1B). However, the peak current of electropolymerization of is suppressed and the ΔE_p of the two pairs of peaks is increased. The

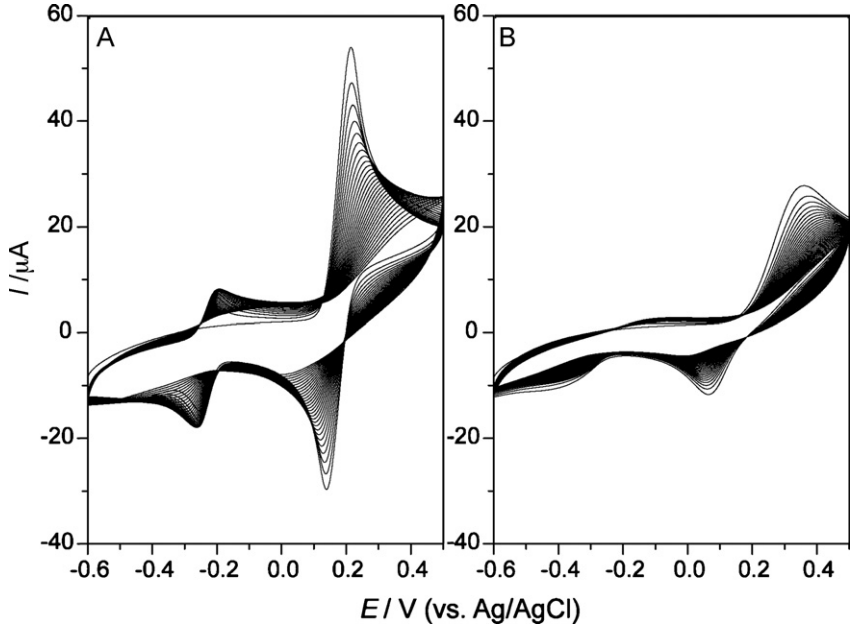


Fig. 1. Electropolymerization of polydopamine film on the (A) bare GCE, and (B) HRP/BLM electrode. Scan rate: 100 mV/s.

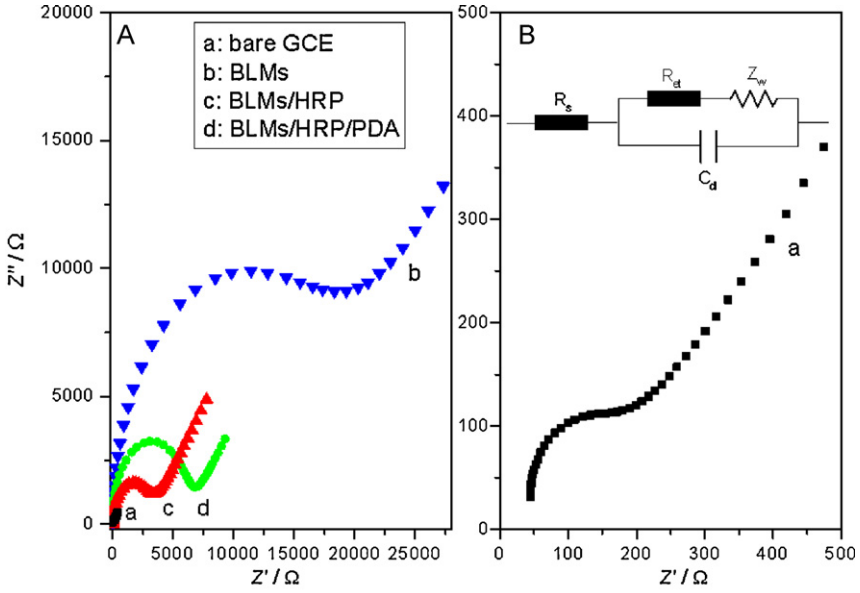


Fig. 2. (A) Nyquist plots of bare GCE (a), BLM (b), HRP/BLM (c), PDA/HRP/BLM (d) in 10 mmol L^{-1} $K_4Fe(CN)_6/K_3Fe(CN)_6$ aqueous solution containing 0.1 mol L^{-1} KCl. (B) An enlargement of the plot of bare GCE.

result shows that the insulating BLM inhibits the electropolymerization of polydopamine.

3.2. Electrochemical impedance spectroscopy of PDA/HRP/BLM electrode

Electrochemical impedance spectroscopy (EIS) is an effective technique for providing useful information about the impedance changes of the electrode surface during the fabrication process. The Nyquist plot of the EIS includes a semicircular portion and a linear portion. The semicircular portion at higher frequencies corresponds to the electron-transfer-limited process and its diameter is equal to the electron transfer resistance (R_{et}), which controls the electron-transfer kinetics of the redox probe at the electrode interface. Meanwhile, the linear part at lower frequencies corresponds to the diffusion process [22]. Fig. 2A shows the Nyquist plots of the EIS of bare GCE (a), BLM (b), HRP/BLM (c), and PDA/HRP/BLM (d) in 10 mmol L^{-1} $K_4Fe(CN)_6/K_3Fe(CN)_6$ (1:1) containing 0.1 mol L^{-1} KCl. Fig. 2B is the enlargement of the plot of bare GCE. Changes of the impedance spectra are observed in the course of stepwise modification of the electrode, and the R_{et} values of the bare GCE, BLM, HRP/BLM, and PDA/HRP/BLM are ca. 0.2, 28, 4.0 and 7.2 kΩ. Compared with the bare GCE, BLM coated on the electrode can block the contact of the redox probe $[Fe(CN)_6]^{3-/4-}$ with the electrode surface, resulting in an obviously increased in R_{et}, which indicates that BLM is formed successfully on the electrode and could further obstruct electron transfer of the redox probe. Compared with the BLM/GCE, an obvious decrease in the interfacial resistance is observed at the HRP/BLM/GCE (curve c). It shows that HRP could insert at least partly into the BLMs or lead to some defect or lesion in

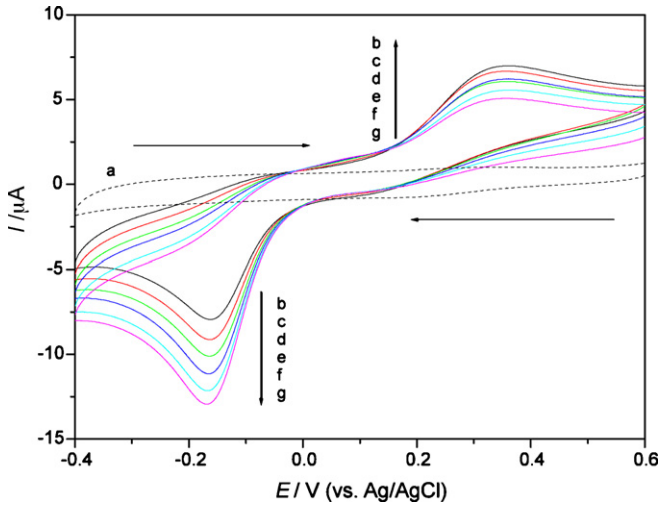


Fig. 3. Cyclic voltammograms of PDA/HRP/BLM electrode in 0.1 mol L^{-1} pH 7.0 PBS (a), 0.1 mol L^{-1} pH 7.0 PBS containing 0.4 mmol L^{-1} HQ (b), 0.1 mol L^{-1} pH 7.0 PBS containing 0.4 mmol L^{-1} HQ and 0.1 (c), 0.2 (d), 0.3 (e), 0.4 (f), 0.5 mmol L^{-1} (g) H_2O_2. Scan rate: 100 mV s^{-1}.

the BLM, resulting in the increased permeability of the membrane. When a PDA film is further electropolymerized on the surface of HRP/BLM/GCE, the R_{et} of PDA/HRP/BLM/GCE is increased from 4.0 to 7.2 kΩ. It indicates that a PDA film is successfully coated on the HRP/BLM and block electron transfer of the redox probe. From the EIS, it can be concluded that PDA/HRP/BLM is fabricated successfully by the stepwise modification of the electrode. The BLM system could supply a biological environment for enzyme to maintain activity and stability.

3.3. Cyclic voltammetric behavior of PDA/HRP/BLM electrode

Fig. 3 shows the cyclic voltammograms of PDA/HRP/BLM electrode in 0.1 mol L^{-1} PBS (pH 7.0). In the absence of hydroquinone (HQ) and H_2O_2, no response of the enzyme electrode is observed. The electrode displays a low background current. When 0.4 mmol L^{-1} HQ is added to the PBS, the cyclic voltammogram shows a couple of reduction and oxidation peaks of HQ. With increasing scan rate, the ΔE_p increases and the anodic and cathodic peak currents increase linearly with the square root of the scan rate from 10 to 500 mV s^{-1}, indicating a typical quasi-reversible behavior and no adsorption of the oxidized and reduced forms of HQ on the enzyme electrode surface. When H_2O_2 is added to the substrate solution, the reduction peak current increases and the oxidation peak current decreases dramatically in the cyclic voltammogram. The results show that BLM film cannot only preserve the bioactivity of HRP immobilized in the membrane, but also provide a channel for the electron transfer between HRP and HQ at the surface of PDA/HRP/BLM electrode.

3.4. Effects of pH, HQ concentration, and applied potential on the response of PDA/HRP/BLM electrode

The effect of pH on the response of the H_2O_2 biosensor depends on two factors: the activity of HRP and the stability of BLM. The relationship between the catalytic peak current and pH from 5.5 to 8.5 is studied. The biosensor shows the maximum peak current at pH 7.0. So phosphate buffer solution (pH = 7.0) is selected as working solution.

The concentration of mediator also plays an important role on the catalytic peak current of the biosensor. The effect of HQ concentration on the response current of biosensor is studied. The H_2O_2 electrocatalytic reduction current increases rapidly in the HQ concentration from 0 to 0.4 mmol L^{-1}, and reaches a plateau at 0.4 mmol L^{-1}. Further increase of HQ concentration does not increase the response current much. So 0.4 mmol L^{-1} HQ is selected for H_2O_2 measurement to avoid the interference of catalytic peak current by mediator.

The effect of applied potential on the response of the H_2O_2 is investigated from −50 to −300 mV. The H_2O_2 electrocatalytic reduction current increases rapidly under the potential from −50 to −150 mV, and reaches a plateau at −150 mV. Further decrease of potential from −150 to −300 mV does not increase the response current much. In order to obtain high response current and avoid the interfering of the interference substances, the electrode potential is maintained at −150 mV for amperometric measurements.

3.5. Amperometric response of PDA/HRP/BLM electrode

The electrocatalytic reduction of H_2O_2 on PDA/HRP/BLM electrode is studied by amperometry. Fig. 4A shows the typical steady state current-time response of the biosensor. The response time of the biosensor is less than 5 s until steady state value is obtained. The response current increases with the increase of H_2O_2 concentration. The calibration curve gives a linear range of H_2O_2 concentration from 2.5×10^{-7} to 3.1×10^{-3} mol L^{-1} with a correlation coefficient of 0.9992 (Fig. 4B). The detection limit is calculated 1.0×10^{-7} mol L^{-1} (S/N = 3) and the relative standard deviation is 2.8%. The sensitivity of the PDA/HRP/BLM electrode

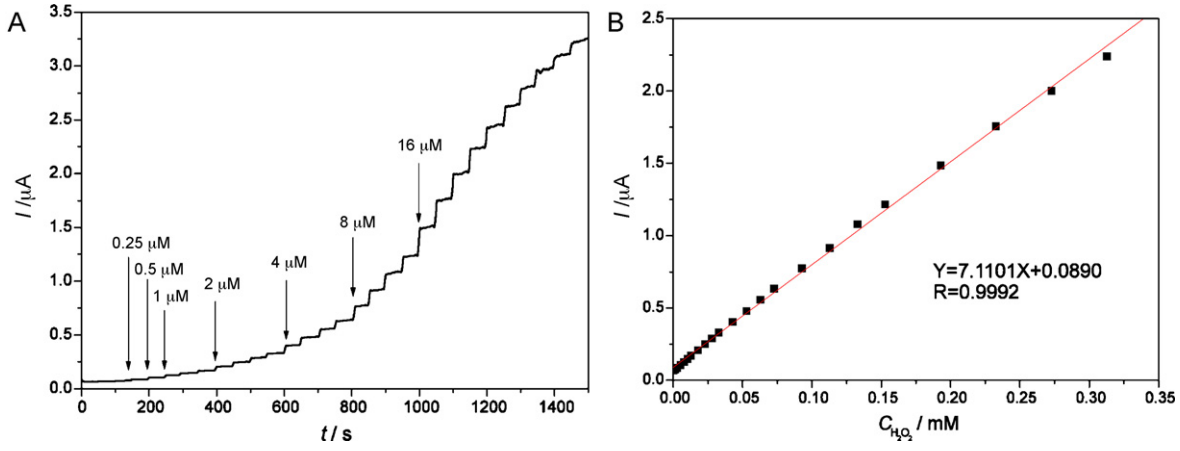


Fig. 4. (A) Chronoamperometric response of the biosensor to successive additions of H_2O_2 in 0.1 mol L^{-1} PBS (pH = 7.0) containing 0.4 mmol L^{-1} HQ. (B) The calibration curve of the currents on the concentration of H_2O_2.

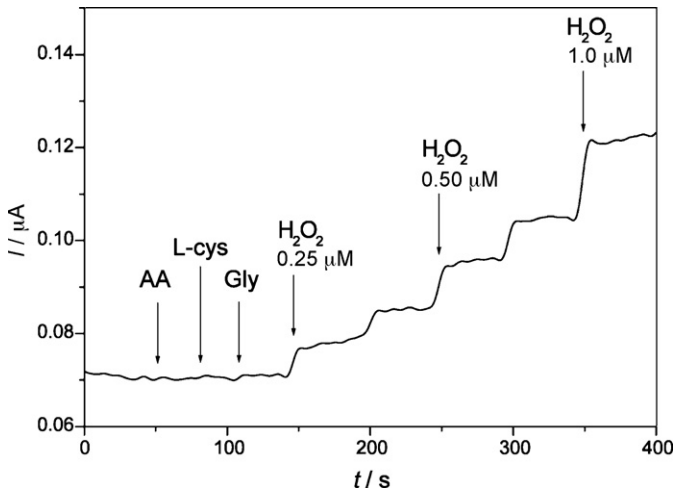


Fig. 5. The amperometric response of 10 μmol L^{-1} ascobic acid (AA), L-cysteine (L-cys), glycine (Gly) and different concentrations of H_2O_2 on the PDA/HRP/BLM modified electrode. Potential applied: −0.15 V.

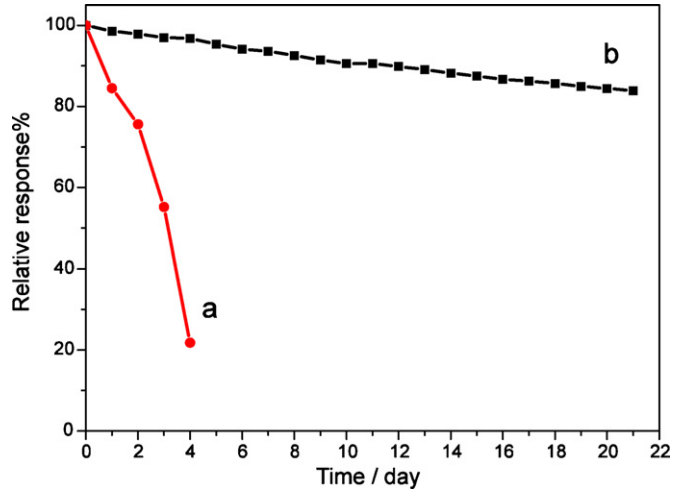


Fig. 6. Storage stability of HRP/BLM (a) and PDA/HRP/BLM (b).

is superior to that of the well-established H_2O_2 BLM biosensors [23,24].

3.6. Interference of PDA/HRP/BLM electrode

Interferential effect is investigated by testing the response of the biosensor to ascobic acid, L-cysteine, and glycine (Fig. 5). The three compounds are the main substances that interfere with the detection of H_2O_2. It shows that 40 folds of the three interfering substances do not affect the measurement of H_2O_2. The excellent interference resistibility can be due to the coverage of a polydopamine film on the BLM biosensor. The polydopamine film hinders the interfering substances from diffusing to the electrode surface.

3.7. Reproducibility and stability of PDA/HRP/BLM electrode

The reproducibility and stability of the PDA/HRP/BLM electrode are examined by amperometric measurement of 0.5 mmol L^{-1} H_2O_2 in 0.1 mol L^{-1} PBS (pH = 7.0) containing 0.4 mmol L^{-1} HQ. Six biosensors are fabricated based on the same construction to examine their amperometric responses, and the relative standard derivation (RSD) is 3.2%. The repeatability of one electrode is also examined by successive measurements. The electrocatalytic reduction current decreases only 2.5% after 20 successive measurements. The storage stability of the biosensor is studied by storing it at 4 °C and measuring once a day over a period of 21 days (Fig. 6). The electrocatalytic activity of PDA/HRP/BLM biosensor retains 84% of its original response within 3 weeks and the relative standard deviation is 4.5%. In contrast, the response of HRP/BLM biosensor decreases dramatically and loses 80% of its initial response within 4 days.

4. Conclusion

In summary, a new kind of polydopamine film is electropolymerized on the surface of BLM to prepare PDA/HRP/BLM biosensor. The immobilized HRP is used to construct H_2O_2 biosensor in the presence of HQ as mediator. To determine the optimum conditions for the biosensor, the effects of experimental conditions such as solution pH, HQ concentration, and applied potential are investigated. The resulting biosensor exhibits excellent performance for the determination of H_2O_2. A linear relationship between 2.5×10^{-7} and 3.1×10^{-3} mol L^{-1} is obtained under the optimal experimental conditions, with a detection limit of 1.0×10^{-7} mol L^{-1}. The selectivity, repeatability, and stability of the biosensor are also investigated with satisfactory results. It shows that the reproducibility and stability of PDA/HRP/BLM biosensor are greatly enhanced comparing with that of HRP/BLM biosensor. On the PDA/HRP/BLM biosensor, the surface of HRP/BLMs is coated with an adhesive film polydopamine, which can retain the structure of BLMs and avoid the leakage of HRP enzyme from the BLM. The biocompatible polydopamine also protect the HRP enzyme from deactivating. Therefore, the electrocatalytic activity of PDA/HRP/BLMs biosensor can be remained for a long time.

Acknowledgements

This work is financially supported by National Natural Science Foundation of China (No. 20965003), Key Technology R&D Program of Jiangxi Province (Grant No. 2010BGA01800), Key Science and Technology Research Program from Ministry of Education of China (Grant No. 210112), and the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry.

References

[1] P. Buehlmann, H. Aoki, K.P. Xiao, S. Anemiya, K. Tohda, Y. Umezawa, Electroanalysis 10 (1998) 1149–1158.
[2] H. Kiefer, B. Klee, E. John, Y.D. Stierhof, F. Jahnig, Biosens. Bioelectron. 6 (1991) 233–237.
[3] Z.A. Xu, X. Chen, S.J. Dong, Trends Anal. Chem. 25 (2006) 899–908.
[4] W.M. Huang, J.B. Jia, Z.L. Zhang, X.J. Han, J.L. Tang, J.G. Wang, S.J. Dong, E.K. Wang, Biosens. Bioelectron. 18 (2003) 1225–1230.
[5] P. Muller, D.O. Rudin, H.T. Tien, W.C. Wescott, Nature 194 (1962) 979–980.
[6] T. Martynski, H.T. Tien, Bioelectrochem. Bioenergy 25 (1991) 317–324.
[7] H.T. Tien, A.L. Ottova, Electrochim. Acta 43 (1998) 3587–3610.
[8] E. Sackman, Science 271 (1996) 43–48.
[9] C.G. Siontorou, A.M.O. Brett, D.P. Nikolelis, Talanta 43 (1996) 1137–1144.
[10] D.P. Nikolelis, T. Hianik, G.P. Nikoleli, Electroanalysis 22 (2010) 2747–2763.
[11] J.L. Wang, F.A. Wang, H.J. Chen, X.H. Liu, S.J. Dong, Talanta 75 (2008) 666–670.
[12] H. Lee, S.M. Dellatore, W.M. Miller, P.B. Messersmith, Science 318 (2007) 426–430.
[13] J.H. Waite, M.L. Tanzer, Science 212 (1981) 1038–1040.
[14] T.A. Morris, A.W. Peterson, M.J. Tarlov, Anal. Chem. 81 (2009) 5413–5420.
[15] W.H. Zhou, C.H. Lu, X.C. Guo, F.R. Chen, H.H. Yang, X.R. Wang, J. Mater. Chem. 20 (2010) 880–883.
[16] B. Fei, B.T. Qian, Z.Y. Yang, R.H. Wang, W.C. Liu, C.L. Mak, J.H. Xin, Carbon 46 (2008) 1795–1797.
[17] X.B. Yin, D.Y. Liu, J. Chromatogr. A 1212 (2008) 130–136.
[18] Y.C. Fu, P.H. Li, T. Wang, L.J. Bu, Q.J. Xie, X.H. Xu, L.H. Lei, C. Zou, J.H. Chen, S.Z. Yao, Biosens. Bioelectron. 25 (2010) 1699–1704.
[19] Y.M. Tan, W.F. Deng, Y.Y. Li, Z. Huang, Y. Meng, Q.J. Xie, M. Ma, S.Z. Yao, J. Phys. Chem. B 114 (2010) 5016–5024.

[20] Y.C. Fu, P.H. Li, L.J. Bu, T. Wang, Q.J. Xie, X.H. Xu, L.H. Lei, C. Zou, J.H. Chen, S.Z. Yao, J. Phys. Chem. C 114 (2010) 1472–1480.
[21] K. Liu, W.Z. Wei, J.X. Zeng, X.Y. Liu, Y.P. Gao, Anal. Bioanal. Chem. 385 (2006) 724–729.
[22] J.J. Feng, G. Zhao, J.J. Xu, H.Y. Chen, Anal. Biochem. 342 (2005) 280–286.
[23] Z.Y. Wu, B.Q. Wang, Z.L. Cheng, X.R. Yang, S.J. Dong, E.K. Wang, Biosens. Bioelectron. 16 (2001) 47–52.
[24] J.L. Tang, B.Q. Wang, Z.Y. Wu, X.J. Han, S.J. Dong, E.K. Wang, Biosens. Bioelectron. 18 (2003) 867–872.