Biosensors and Bioelectronics 24 (2009) 1527–1530

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Short communication

A glucose biosensor based on Prussian blue/chitosan hybrid film

Xueying Wang[a,b], Haifang Gu[a], Fan Yin[b], Yifeng Tu[a,*]

[a] Institute of Analytical Chemistry, Department of Chemistry, Dushu-Lake Campus, Suzhou University, Suzhou 215123, PR China
[b] Department of Chemistry, Changshu Institute of Technology, Changshu 215500, PR China

ARTICLE INFO

Article history:
Received 25 September 2008
Accepted 26 September 2008
Available online 14 October 2008

Keywords:
Glucose biosensor
Prussian blue
Chitosan
Electrodeposition

ABSTRACT

Based on electrodeposition of Prussian blue (PB) and chitosan (CS) directly on gold electrode, a hybrid film of PB/CS has been prepared. PB in this film shows a good stability compared with pure PB film when it worked in neutral and weak alkalescent solution and can act as redox mediator. It provides the potential application of such film in biosensor fabrication. A glucose biosensor was fabricated by electrodepositing glucose oxidase (GOD)/CS film on this PB/CS modified electrode. The optimum experimental conditions of biosensor for the detection of glucose have been studied in detail. Under the optimal conditions, a linear dependence of the catalytic current upon glucose concentration was obtained in the range of 2×10^{-6} to 4×10^{-4} M with a detection limit of 3.97×10^{-7} M. The resulting biosensor could be applied to detect the blood sugar in real samples without any pretreatment.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

Based on the enzymatic oxidation for zymolyte, biosensors have attracted special interest for researchers owing to their practical advantages, such as operation simplicity, lower expense of fabrication, higher selectivity and suitability for real time detection (Luo and Do, 2004; Zhao et al., 2005). Usually, the detection may be accomplished by monitoring the formation of hydrogen peroxide (Sulak et al., 2006). However, the high positive working potentials required for the direct electrocatalysis of hydrogen peroxide on bare gold electrode, might lead to interferences from reducing species such as ascorbic acid, uric acid and so on (Wu et al., 2007). To solve this problem, a redox mediator would be adopted to improve the electron transfer between enzyme or coenzyme and electrode which could reduce the positive working potential.

The Prussian blue (PB) is an efficient redox mediator for selective detection of hydrogen peroxide in the presence of oxygen and other interferents and has been applied in the construction of a large number of oxidase enzyme-based biosensors for clinical, environmental and food analysis (Karyakin et al., 1995, 2000; Li et al., 2003). But unfortunately, the PB was not stable on electrode surface when it worked at neutral pH (Ricci et al., 2003), which limited its use in biosensor fabrication. Thus, it is necessary to find a suitable method to increase the operational stability of PB film in neutral pH.

Chitosan is a non-acetylated or partially deacetylated chitin. It can be found in the fungal cell wall and the exoskeletons of lots of arthropods such as crabs and shrimps. Because of some primary amino groups of about 6.3 of pK_a (Sorlier et al., 2001), it acts as a water-soluble polyelectrolyte at lower pH solutions (<pK_a) due to most of the amino groups were protonated. When the pH is higher than pK_a, the amino groups are deprotonated then chitosan would become insoluble. Based on its excellent film forming ability, biocompatibility, nontoxicity, high mechanical strength, cheapness and a susceptibility to chemical modifications, it has been extensively used for immobilization of enzymes and to construct the amperometric biosensors by cross-linking with enzyme or other substances (Chen and Gorski, 2001; Chen et al., 2003; Miao and Tan, 2001; Wang et al., 2002, 2003; Wei et al., 2002, 2003).

However, the covalent cross-linking for enzyme might lead to the partly loss of activity, and the manual coating of the electrode is in difficulty to control the thickness of films. Direct ionotropic gelation (Wei et al., 2003) can immobilize enzyme on electrode with chitosan and the immobilized enzyme retains high activity, but the stability of this biocomposite film is rather poor, and the thickness of the film is still uncontrollable.

Electrochemical deposition has been reported recently as a method suitable for selective deposition of films with controllable thickness (Deepa et al., 2003). Other substances such as gold nanoparticles (Bharathi et al., 1999) and enzymes (Bharathi and Nogami, 2001) can be effectively incorporated to form the biocomposites during the electrodeposition. Chitosan hydrogel can also be electrochemically deposited onto electrodes (Wu et al., 2002, 2003; Fernandes et al., 2003) tightly and retain its natural properties.

* Corresponding author. Tel.: +86 13812768378; fax: +86 51265101162.
E-mail address: tuyf@suda.edu.cn (Y. Tu).

0956-5663/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2008.09.025

A new type of glucose biosensor was constructed in this research based on electrochemical deposition of PB/CS hybrid film on a gold electrode as a mediator for the detection of hydrogen peroxide. The PB showed better stability in neutral and weak alkalescent medium when it was hybridized with CS. And then the GOD was immobilized onto this modified electrode by synchronous electrodeposition with also CS instead of the cross-linking covalent reaction. The experimental conditions related to the electrodeposition and characteristics of biocomposite and the performance of the resulting biosensors were investigated in detail.

2. Experiments

2.1. Reagents

The glucose oxidase(E.C.1.1.3.4) and the chitosan (MW 1.9–3.1 × 10^5, minimum 85% deacetylation) were obtained from Sigma Chemical Co. Glucose was purchased from Beijing Chemical Reagent Co. (Beijing, China). All other chemicals were of analytical grade. Phosphate buffer solution (PBS, 1/15 M) consisted of K_2HPO_4, KH_2PO_4 and 0.1 M KCl was employed as supporting electrolyte. The double-distilled water was used throughout.

2.2. Instruments

A standard three-electrode electrochemical cell was used in all the cases with a Pt disk electrode (2.0 mm diameter) as the counter electrode, a saturated calomel electrode (SCE) as reference electrode and a gold disk electrode (2.0 mm diameter, CHI101, CH Instruments, USA) as working electrode. The cyclic voltammetry and chronoamperometry measurements were carried out on a CHI660C electrochemical workstation (Shanghai Chenhua Instrument Co., China). The electrochemical impedance spectroscopy (EIS) was obtained using a PARSTAT 2273 electrochemical workstation (PE Co., USA). EIS was performed in the frequency range from 10 kHz to 100 mHz. The bias voltage, a DC potential applied to the working electrode relative to the SCE, was set to 0.15 V, while the amplitude of the AC signal applied to the working electrode was set to 10 mV during all experiments. Fourier transformation infrared spectroscopy was recorded with a Nicolet380 FT-IR spectrometer (Thermo Fisher Scientific Co., USA).

2.3. Electrode preparation

2.3.1. Preparation of PB/CS hybrid film

A 1.0% chitosan solution was prepared by dissolving 1.0 g of chitosan flakes into 100 ml of 1.0% acetic acid and stirred for 3 h at room temperature until complete dissolution. The solution was stored in refrigerator when not in use.

A gold disk electrode was polished with Al_2O_3 powder (0.03 μm), and then it was cleaned successively with 2 M NaOH, 1 M H_2SO_4, 95% ethanol and double-distilled water for 5 min by ultrasonic. It was further modified in a solution containing 2.5 mM $FeCl_3$, 2.5 mM $K_3[Fe(CN)_6]$, 0.1 M KCl, 0.01 M HCl and 0.01% CS with a constant potential of 0.4 V (vs. SCE) for electrodeposition for 300 s. After that, the electrode was cyclic scanned in the potential range from −0.2 to 0.5 V (vs. SCE) at the scan rate of 50 mV/s until the CV curve was stable.

2.3.2. Preparation of GOD/CS hybrid film

The GOD/CS hybrid film was deposited electrochemically on that prepared PB/CS modified electrode in a buffer (pH 6.5, 1/15 M phosphate, 0.1 M KCl, 5 mg/ml GOD and 0.1% CS) applying constant potential of 0.4 V (vs. SCE) for 150 s. The whole period to accomplish the preparing of a biosensor could be limited in 30 min. And then it was immersed in a phosphatic buffer at 4 °C before use.

3. Results and discussion

3.1. Characterization of PB/CS and GOD/CS-PB/CS film

FT-IR, EIS and cyclic voltammetry were applied to character the formation and properties of the films.

The specimens for IR measurement were prepared by gathering up the deposited materials from electrode surface. Their FT-IR spectra and the attribution of the bands could be seen in supplementary file (SFig. 1 and STable 1). In primary, the band around the wave number of 3400 cm^{-1} which could be attributed to –OH or –NH groups wherever in CS or GOD appeared both on spectrum of PB/CS or GOD/PB/CS, the typical absorbance band attributed to stretching vibration of –C=O group (around 1640 cm^{-1}) also exist both on those spectra, the bending vibration of amide (around 1560 cm^{-1}) can be still observed in spectrum of GOD/PB/CS in spite of it was too weak to be affirmed in PB/CS composite, and also the stretching vibration of –CN group which was contained in molecule of PB around 2080 to 2040 cm^{-1} appeared both on spectrum of PB/CS and GOD/PB/CS. All of aforementioned facts have indicated that the specimens of deposited composite showed all of proper absorbant bands of CS and PB for CS/PB film and CS, PB and GOD for PB/CS/GOD film. It suggests that the GOD was effectively immobilized onto the PB/CS hybrid film and its essential feature of the native structure was not distinguishably changed after been immobilized onto the hybrid film.

Also the EIS is a well-known effective method for studying the interface properties (Shervedani et al., 2006). In there, the semicircle diameter at higher frequency in the Nyquist plot could be used to describe the interface properties especially the changes when substances covered on electrode. With 1 mM$Fe(CN)_6^{3-/4-}$ as the probe, the electrodes showed typical EIS with different parameters (see SFig. 2 in supplemental materials). It has been found that the diameter of the Nyquist circle decreased when PB/CS was deposited on bare electrode, which means that PB/CS film can increase the electron transfer rate. Otherwise, after further depositing of GOD/CS film on it, EIS shown a larger semicircle than bare Au electrode, means a slowered electron transference. It reveals that additional CS and GOD molecules hindered the probe to contact with electrode.

Then the cyclic voltammetry was used to characterize the electrochemical behaviour of PB which coexisted with CS on electrode surface. With increasing scan rate from 50 to300 mV/s, the anodic and cathodic peak currents increased linearly (see SFig. 3 in supplemental materials), indicating a surface-controlled process (Laviron, 1979; Laviron and Roullier, 1998).

3.2. Stability of PB/CS hybrid film in neutral and alkalescent solution

Studies of the effect from acidity on modified electrodes were carried out to evaluate the electrochemical behavior and the stability with pH changes. It is well known that PB is unstable and dissolvable at pH values above 7.0 (Karyakin, 2001), suggesting a limited use of PB modified electrode in acidic solution (Boyer et al., 1990; Wang et al., 1999). Fig. 1 shows the response of voltammetric peak current of pure PB modified electrode (group B) and PB/CS modified electrode (group A) upon the pH of buffer solution. Pure PB modified electrode would lose about 50% of its efficiency if the pH rose to 6.0 and the redox currents would be lower at pH 7.0 and disappeared at pH 8.0, indicating that pure PB film was unstable

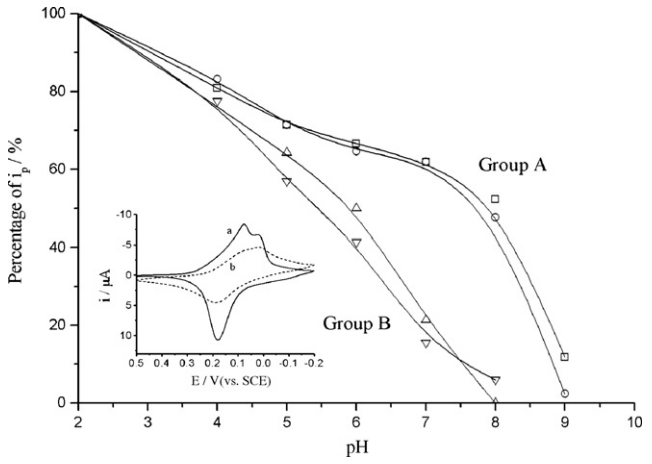

Fig. 1. CV peak currents of pure PB film (Group B) and PB/CS hybrid film (Group A) dependent on pH. Insert is the CV curves at pH 6.0.

and dissolved. The PB/CS hybrid film can improve the stability of PB during the raise of pH. Fig. 1 (group A) shows it kept the activity in neutral and weak alkalescent solution of higher pH than 8.0. It still gave higher, well-defined and reversible redox peaks, would provide the potential application of such film in biosensor fabrication because that the optimum acidity for many enzymes was within the pH range of 6.5–8.0.

3.3. Electrocatalysis of PB/CS film to reduction of H_2O_2

With the addition of H_2O_2 into the electrochemical cell using a PB/CS/Au electrode as working electrode in 1/15 M PBS of pH 7.0, the cathodic peak and the anodic peak, on its cyclic voltammogram, increased and decreased, respectively (Fig. 2, curve c). Compared with the CV curve in the absence of H_2O_2 (Fig. 2, curve b), it is characteristic of an electrochemically catalytic reaction of PB to H_2O_2. Correspondingly, no electrochemical signal upon H_2O_2 on bare gold electrode can be observed in the interesting potential range (Fig. 2, curve a). It is reasonable to consider that the catalytic peak comes from the interaction between PB and H_2O_2. This result shows that PB/CS film can act as an effective catalyst to the reduction of H_2O_2.

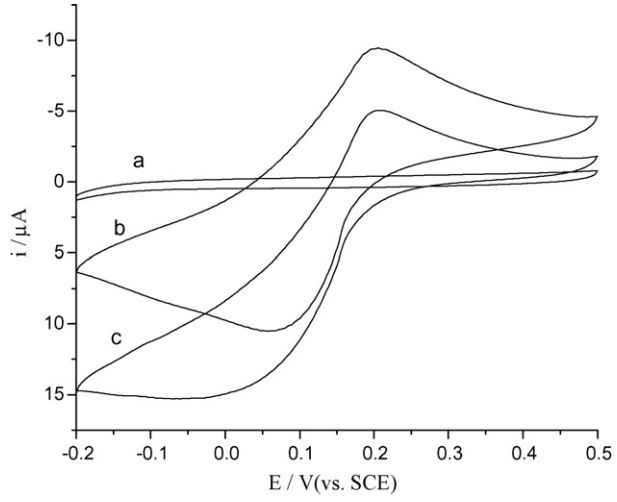

Fig. 2. Cyclic voltammograms of (a) Au electrode in the presence of 10 mM H_2O_2, PB/CS modified Au electrode in the (b) absence and (c) presence of 10 mM H_2O_2, scan rate = 50 mV s^{-1}, 1/15 M PBS buffer (pH 7.0).

3.4. Optimization of biosensor's working conditions

The GOD/CS-PB/CS modified electrode was prepared according to Section 2.3. This modified electrode can be used as a glucose biosensor. The effect of the working potential on the amperometric response of the biosensor was studied between −0.2 and +0.2 V. The result is shown that the maximum response was reached at −0.1 V and it was selected as the working potential (see curve A of SFig. 4 in supplemental materials).

The experimental results have shown that the optimum response of the biosensor upon the acidity of buffer was obtained in pH range from 4 to 7 (see curve B of SFig. 4 in supplemental materials), which is lower than that in reference (Yang et al., 2004). This phenomenon may be caused by buffer effect of CS because it has great deal of amino groups, which can provide a suitable pH microenvironment for GOD. In this experiment, pH value of 6.0 was chosen for the detection of glucose.

Current responses of the biosensor at different temperatures were determined from 10 to 50 °C. The response increased with the increase of temperature, reaching a maximum value at about 40 °C (see curve C of SFig. 4 in supplemental materials). When the temperature was higher than 40 °C, the current decreased rapidly. It may be caused by the denaturation of the enzyme. Thus, the temperature of 35 °C was chosen for the detection of glucose.

3.5. Amperometric response of the biosensor to glucose

Fig. 3 displays the chronoamperometric response of the biosensor upon the successive injection of glucose into electrochemical cell with stirring. The reduction current increased steeply and then reached 95% of the steady-state current in less than 10 s. The results implied that the biosensor's response was very fast. The linear response range of the biosensor to glucose concentration is from 2×10^{-6} to 4×10^{-4} M with a correlation coefficient of 0.9970. The regression equation is i (μA) = 0.058 + 2.57$C_{glucose}$ (mM). The detection limit can be estimated for 3.97×10^{-7} M defined from a signal/noise ratio of 3. When the concentration of glucose is higher, the current response would gradually reach to a stable value (i_o) for about 1.67 μA, so it is conceivable to evaluated the Michaelis constant (K_m) for 3.73 mM. This datum is smaller than reported ones of dissociative or immobilized GOD. It indicates that the CS is an excellent immobilizing matrix and activity holder for enzyme.

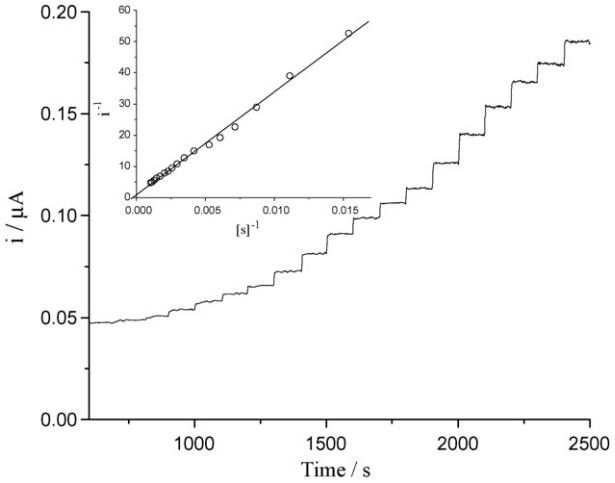

Fig. 3. Typical chronoamperometric response of the biosensor to successive injection of glucose. Inset the Lineweaver–Burk plot of the biosensor.

There is no significant change of the response current to be observed by adding 0.3 mM uric acid or 0.06 mM ascorbic acid into glucose solution, indicated that the obtained biosensor has good anti-interferent ability. The reproducibility of this biosensor was evaluated with relative standard deviation (R.S.D.) of 6.58% ($n = 6$) for 0.1 mM of glucose. The stability of the resulting biosensor was investigated by discontinuous amperometric measurement after stored in 4 °C for weeks and just found slight degeneration for the current will retain about 90% of its initial value.

This glucose biosensor could be applied to assay the real blood sugar. Three fresh serum samples were supplied by the Affiliated Hospital of Suzhou University. Every 0.1 ml of serum samples was diluted with 1/15 M phosphate buffer (pH 6.0) to 5 ml. By using standard addition method, the blood sugar indexes of three samples were obtained for 5.9, 5.7 and 4.9, respectively, and the recovery values were in the range of 87.6–102.6% (the detailed data could be seen in STable 2 in supplemental materials).

4. Conclusion

A PB/CS hybrid film has been prepared by electrodepositing it directly on gold electrode. PB in this hybrid film was more stable than pure PB on electrode during the raise of pH. An amperometric glucose biosensor has been fabricated by further electrodeposition of glucose oxidase composited with CS on this modified electrode. From the smaller Michaelis constant, it can be concluded that the CS is an excellent immobilizing matrix and activity holder for enzyme. According to this research, the PB/CS hybrid film shows the potential application in biosensor design for the detection of real blood sample.

Acknowledgement

This work is supported by the NSFC (20275025, 20675055).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2008.09.025.

References

Bharathi, S., Joseph, J., Lev, O., Lev, Z., 1999. Solid-State Lett. 2, 284–287.
Bharathi, S., Nogami, M., 2001. Analyst 126, 1919–1922.
Boyer, A., Kalcher, K., Pietsch, R., 1990. Electroanalysis 2, 155–161.
Chen, L., Gorski, W., 2001. Anal. Chem. 73, 2862–2868.
Chen, X., Jia, J., Dong, S., 2003. Electroanalysis 15, 608–612.
Deepa, P.N., Kanungo, M., Claycomb, G., Sherwood, P.M.A., Collinson, M., 2003. Anal. Chem. 75, 5399–5405.
Fernandes, R., Wu, L.-Q., Chen, T., Yi, H., G.W., Rublo, V., Ghodssi, R., Bentley, W.E., Payne, G.F., 2003. Langmuir 19, 4058–4062.
Karyakin, A.A., 2001. Electroanalysis 13, 813–819.
Karyakin, A.A., Karyakina, E.E., Gorton, L., 2000. Anal. Chem. 72, 1720–1723.
Karyakin, A.A., Gitelmacher, O.V., Karyakina, E.E., 1995. Anal. Chem. 67, 2419–2423.
Laviron, E., 1979. J. Electroanal. Chem. 101, 19–28.
Laviron, E., Roullier, L., 1998. J. Electroanal. Chem. 443, 195–207.
Luo, Y.-C., Do, J.-S., 2004. Biosens. Bioelectron. 20, 15–23.
Li, J.P., Peng, T.Z., Peng, Y.Q., 2003. Electroanalysis 15, 1031–1037.
Miao, Y., Tan, S.N., 2001. Anal. Chim. Acta 437, 87–93.
Ricci, F., Amine, A., Palleschi, G., Moscone, D., 2003. Biosens. Bioelectron. 18, 165–174.
Shervedani, R.K., Mehrjardi, A.H., Zamiri, N., 2006. Bioelectrochemistry 69, 201–208.
Sorlier, P., Denuzi'ere, A., Viton, C., Domard, A., 2001. Biomacromolecules 2, 765–772.
Sulak, M.T., Gokdogan, O., Gulce, A., Gulce, H., 2006. Biosens. Bioelectron. 21, 1719–1726.
Wang, G., Xu, J.J., Chen, H.Y., Lu, Z.H., 2003. Biosens. Bioelectron. 18, 335–343.
Wang, G., Xu, J.J., Ye, L.H., Zhu, J.J., Chen, H.Y., 2002. Bioelectrochemistry 57, 33–38.
Wang, J., Zhang, X., Prakash, M., 1999. Anal. Chim. Acta 32, 1739–1749.
Wei, X., Cruz, J., Gorski, W., 2002. Anal. Chem. 74, 5039–5046.
Wei, X., Zhang, M., Gorski, W., 2003. Anal. Chem. 75, 2060–2064.
Wu, L.-Q., Gadre, A.P., Yi, H., Kastantin, M.J., Rublo, V.G.W., Bentley, W.E., Payne, G.F., Ghodssi, R., 2002. Langmuir 18, 8620–8625.
Wu, L.-Q., Yi, H., Li, S., Rublo, V.G.W., Bentley, W.E., Ghodssi, R., Payne, G.F., 2003. Langmuir 19, 519–524.
Wu, B.Y., Hua, S.H., Yin, F., Li, J., Zhao, Z.X., Huang, J.D., Chen, Q., 2007. Biosens. Bioelectron. 22, 838–844.
Yang, Y.H., Yang, H.F., Yang, M.Hi., Liu., Y.L., Shen, G.L., Yu, R.Q., 2004. Anal. Chim. Acta 525, 213–220.
Zhao, W., Xu, J.J., Shi, C.G., Chen, H.Y., 2005. Langmuir 21, 9630–9634.