Fabrication of Tyrosinase Nano-biosensor for Catechol Inspection

Huanan Guan[a※], Benjun Li[b]

[a]College of Food Engineering, Harbin University of Commerce, Harbin 150076

[a]email: guanhuanan3@163.com

Department of Pharmacy, Liaoning medical vocational college, Shenyang 110101

[b]email: lbjlnzy@126.com

Keywords: Encapsulation; Tyrosinase; Biosensor; Phenolic compounds; Liposome

Abstract. A novel tyrosinase (Tyr) biosensor based on liposome bioreactor and chitosan (CS) nano-composite has been developed for the detection of phenolic compounds. The encapsulation efficiency and drug loading content of the Tyr-loaded Liposome-based bioreactors were about 46.35± 0.85% and 41.15±0.95%, respectively. The glassy carbon electrode (GCE) was alternately immersed in CS and Tyr liposome bioreactor (TLB) to assemble bilayer films [(CS/TLB)/GCE]. The presence of Tyr in the biosensor confirmed by scanning electron microscopy (SEM), cyclic voltammetry (CV) and electrochemical measurements. The results indicated that the biosensor was applied to detect catechol with a broad linear range from 0.25 nM to 25μM, the detection limit was brought down to 0.125 nM. The novel biosensor exhibits good repeatability and stability.

1 Introduction

Some of the phenolic compounds have been related with health problems and environmental pollution because of their inherent toxicity [1]. They are often introduced into the environment through industrial wastes from resins and plastics production, wood preservation, petroleum refining, dyes, chemicals, and textiles [2]. Hence, it is of great importance in environment analysis to closely monitor the quantity of these phenolic compounds. Here we reported the establishment of a simple, fast and sensitive method for phenolic compounds detection and measurement.

Enzyme-based biosensors represent potential alternatives to these techniques. In particular, biosensors based on tyrosinase (or polyphenol oxidase, Tyr) have been developed for the determination of phenolic compounds [3,4]. Tyrosinase is a copper-containing monooxygenase enzyme that catalyzes conversion of phenolic substrates to catechol and then oxidized to quinine [5]. Thus, the quinine can be electrochemically reduced to allow amperometric detection of phenol. It is catalyzes two different reactions: hydroxylation of monophenols into diphenols and oxidation of diphenols to quinines. A key factor in the construction of a biosensor is the need to achieve adequate and effective enzyme immobilization. Liposomes have shown great potential as a drug delivery system.

In this paper, we describe the fabrication, characterization and analytical performance of a novel Tyr biosensor based on spherical shells liposome bioreactors and chitosan (CS) membranes by LbL technique. Our approach is based on the encapsulation of all the active reagents of a bioreactor within the liposome environment. Using CS and Tyr liposome bioreactor (TLB) nano-composite films to modify the glassy carbon electrode (GCE) can improve electrocatalytic ability and enhance electron transfer, therefore can obtain high amperometric signals with a corresponding low detection limit.The design of the novel biosensor for the detection of phenolic compounds using catechol as example. The proposed strategy can be extended for the development of other enzyme-based biosensors.

2 Experimental

2.1 Materials

Mushroom tyrosinase, the membrane protein OmpF (porin) and catechol were purchased form

Sigma Chemical Co. (USA). A 5 mg/mL solution of Tyr in buffer phosphate 0.01 M (pH 7.0) was used for the enzyme immobilization. Cholesterol, L-α-phosphatidylcholine from egg yolk (Negative charge) and pyranine were purchased from Sigma. All other reagents were of analytical grade and used without further purification.

2.2 Preparation of liposome bioreactors with entrapped Tyr

The encapsulation of Tyr in liposome was performed following the lipid film's hydration technique as described previously [6]. A thin lipid film was formed by dissolving the lipid mixture (LPC: CH: Tyr: pyranine 2:1:0.1:0.01 molar ratio=standard) in chloroform and subsequent removal of the solvent by rotary evaporation at 37°C to complete dryness. After the lipid film was peeled off completely using gentle hand-shaking movements, the formed vesicles were mixed with 5 μL of a 2 mg/mL stock solution of porin in 1% octyl-POE detergent. The mixture was subjected to 30 freeze-thaw cycles by freezing in liquid nitrogen (-195°C) and thawing at 37°C in a water bath. The sample was then diluted to 1 mL, in 25 mM PBS pH 7.0. Non-encapsulated enzyme was deactivated by adding 2 mg/mL pronase and incubating for 5 h at room temperature.

2.3 Fabrication of CS/TLB nano-composite coated GCE via LbL

The multilayer films were assembled on the GCE surface according to the reported procedures [7]. Tyr solution with a concentration of 5 mg/mL was prepared in 0.1 M PBS pH 7.0. CS solution with a concentration of 5 mg/mL was prepared by dissolving 10 mg CS in 2.0 mL of 0.1 mol/L acetic acid. The cleaned GCE was immersed in CS (Positive charge) for 25 min and rinsed in PBS for 5 min, and evaporating in the air to form a layer of CS membrane (denoted as CS/GCE). The CS/GCE was immersed in Tyr liposome bioreactor (TLB) solution (5 mg/mL, Negative charge) for 15 min at room temperature to deposit the monolayer of TLB on the electrode surface, and washed with the PBS, and evaporating in the air to form a layer of TLB membrane (denoted as (CS/TLB)/GCE).

2.4 Apparatus and measurements

Electrochemical measurements were performed on a LK 98 II electrochemical workstation (Lanlike Instrument Company of Tianjin, China) with a conventional three-electrode system comprising platinum wire as counter electrode, Ag/AgCl electrode as reference electrode and (CS/TLB) modified glassy carbon electrode as working electrode. Unless stated otherwise, electrochemical measurements were carried out in a phosphate buffer solution (PBS, 0.1 M, pH 7.0) at room temperature (25±2°C). After stabilization of the capacitive current, the enzymatic reaction was initiated by addition of amount of phenol standard solutions and the response of the sensor was measured.

3 Results and Discussion

3.1 Characterization of Tyr liposome bioreactor (TLB)

TLB had a mean diameter of 8.5± 1.25 μm and 80% (by volume) of the liposome was below 10 μm with a major proportion of the suspension distributed within the window of 1-12 μm. SEM photographs of typical TLB are shown in Fig. 1. The shape of TLB was found to be spherical and non-aggregated. As shown in Fig. 1A, microspheres will extrude each other due to the elasticity of the capsule wall. Fig. 1B shows the external surface morphology of TLB under high magnification. It also gives the whole view of the complete microcapsule. As can be seen, no obvious defect was found in the capsule wall, and the wall of compact structure is beneficial to enhance the barrier property of the microspheres. It can be concluded that the liposome are successfully entrapped within Try and they can keep intact inside the microspheres after encapsulation. The encapsulation efficiency is defined as the ratio of the encapsulated substance to the total substance, which is an important parameter to evaluate the properties of liposome bioreactor carriers. In our experiment, the encapsulation efficiency of Tyr in TLB can be affected by the concentrations of L-α-phosphatidylcholine and cholesterol and the amount of liposome. The encapsulation efficiency of the Tyr loaded TLB was about 46.35± 0.85%. The drug loading content was about 41.15±0.95% after encapsulated in TLB.

3.2 Electrochemical response of phenol at (CS/TLB)/GCE

The total amount of liposome encapsulated enzyme, incorporated in (CS/TLB)/GCE biosensor (1 mg) system was 0.06 mg which measured by Park's assay. As shown in Fig. 2A, with increasing catechol concentration, the voltammetric response of the enzyme electrode increased. It can conclude that the minimum value of the response to catechol was 10^{-12} M. The peak current observed in the simple electronic voltammetric sensing system reflected the activity of immobilized enzyme, which could be used to detect trace phenolic compounds exposure.

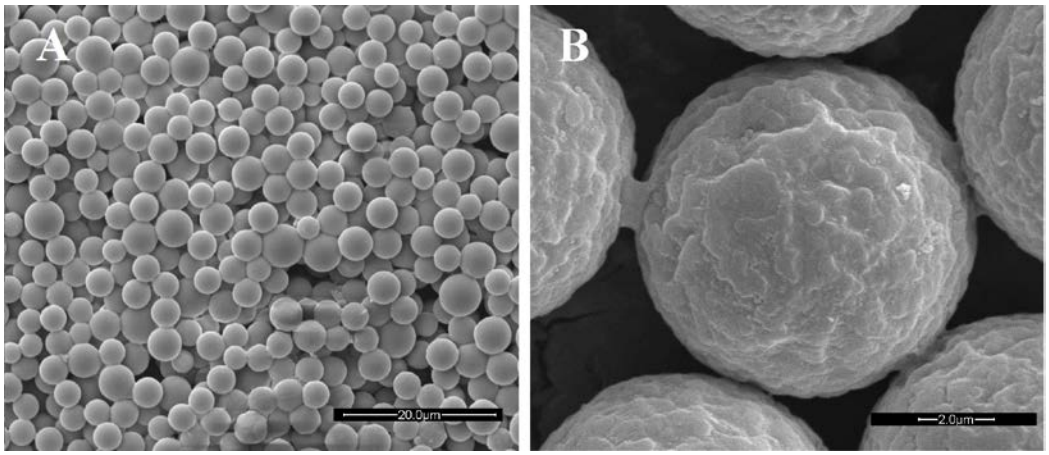

Fig. 1. SEM images of TLB. (A): complete bioreactor with the scale bar 20 μm; (B): complete bioreactor with the scale bar 2 μm.

The linear range spanned from 0.25 nM to 25 μM, with the regression equation: I = (2.37 c+11.45)%, with the correlation coefficients of 0.9877. The detection limit was calculated to be 0.125 nM.

3.3 Stability and reproducibility of the biosensor

In this study, the biosensor was also studied for the Tyr stability at room temperature conditions. The stability of the enzyme was monitored with time to a sample of 5 μM catechol. When not in use, the electrode was stored at room temperature under dry condition. At the same time the activity of the free Tyr in buffer solution stored under exactly the same conditions as the liposome nano-biosensors, was measured for comparison purposes. Fig.2B shows the remaining activity of the enzyme encapsulated in liposome and that of the free Tyr in buffer solution. The liposome encapsulated enzyme retained about 86% of its original response after 5 hours, and decreased gradually to about 73% after 60 hours. After that, the response slightly decreased, possibly due to the dissolution of the enzyme from the electrode, or its possible degradation over time.

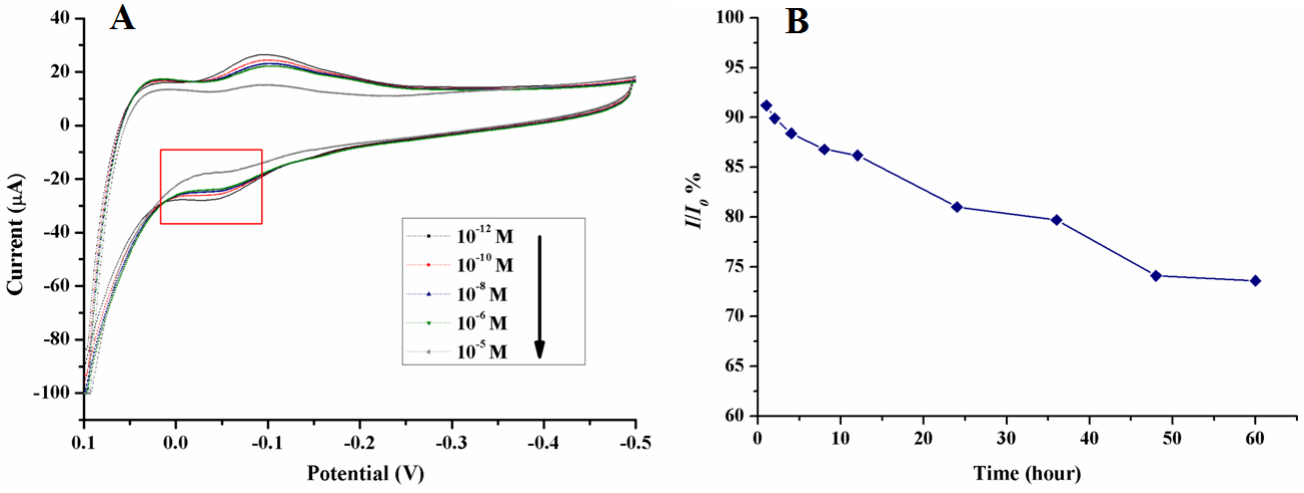

Fig. 2. Effect of different concentrations of catechol on (CS/TLB)/GCE (A); Stability of Tyr encapsulated in biosensor at room temperature (B).

The reproducibility of the biosensor was studied by determining the response to 5 mM catechol at eight different electrodes. The relative standard deviation (R.S.D.) eight successive assays of intra-assay and inter-assay were found to be 3.9 and 5.6%, respectively, indicating acceptable reproducibility. It also suggested that the developed biosensor had high selectivity and little interference from those electroactive substances. The good reproducibility may be due to the fact that the biosensors were fabricated by self-assembly technique.

4 Conclusion

This study has demonstrated the feasibility of developing a conducting liposome bioreactor based biosensor for monitoring phenolic compounds in aqueous medium. The constructed copolymer network incorporating TLB provided a biocompatible microenvironment around the enzyme molecules to stabilize their biological activity and prevented them from leaking out of the interface, and CS membrane as carrier material could absorb more TLB. Additionally, it has confirmed that the conducting bioreactor can be utilized as a suitable matrix for the immobilization of enzyme, tyrosinase. The biosensor exhibits fast response, high sensitivity and stability for the amperometric detection of phenolic compounds because of the high loading of tyrosinase and the rapid electron transfer between the enzymatically produced quinones and the prepared electrode. The biosensor exhibited high sensitivity and stability for the amperometric detection of phenolic compounds. The proposed strategy can be extended for the development of other enzyme-based biosensors.

Acknowledgement

In this paper, the research was sponsored by the Scientific Research Fund of Heilongjiang Provincial Education Department (No. 12531152).

References

[1] J. Zhang, J.P. Lei, Y.Y. Liu, J.W. Zhao, H.X. Ju, Highly sensitive amperometric biosensors for phenols based on polyaniline-ionic liquid-carbon nanofiber composite [J], Biosens. Bioelectron. 2009, 24: 1858-1863.

[2] S.E. Stanca, I.C. Popescu, L. Oniciu, Biosensors for phenol derivatives using biochemical signal amplification [J], Talanta, 2003, 61: 501-507.

[3] Q. Ameer, S.B. Adeloju, Development of a potentiometric catechol biosensor by entrapment of tyrosinase within polypyrrole film [J], Sensors and Actuators B, 2009, 140: 5-11.

[4] K. Zachariah, H.A. Mottola, Continuous-flow determination of phenol with chemically immobilized polyphenol oxidase (tyrosinase) [J], Anal. Lett. 1989, 22: 1145-1158.

[5] L.Q. Wu, T. Chen, K.K. Wallace, R.V. Duhalt, G.F. Payne, Enzymatic coupling of phenol vapors onto chitosan, Biotechnol. Bioeng. 2001, 76: 325-332.

[6] V. Vamvakaki, D.Fournier, N.A. Chaniotakis, Fluorescence detection of enzymatic activity within a liposome based nano-biosensor [J], Biosens Bioelectron. 2005, 21: 384-388.

[7] D.D. Tuoro, M. Portaccio, M. Lepore, F. Arduini, D. Moscone, U. Bencivenga, D.G. Mita, An acetylcholinesterase biosensor for determination of low concentrations of Paraoxon and Dichlorvos [J], New Biotech. 2011, 29: 132-138.