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An amperometric biosensor fabricated from electro-co-deposition of sodium alginate and horseradish peroxidase

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ABSTRACT

A convenient and effective strategy for fabrication of hydrogen peroxide biosensor based on sodium alginate (SA) and polyvinyl butyral (PVB) as matrices was reported in this paper. The horseradish peroxidase (HRP) and SA were electro-co-deposited onto the surface of gold electrode, and the HRP–SA/Au electrode was further coated with PVB. The interaction between HRP and SA was characterized by UV–vis absorption spectroscopy, and the fabricating process of biosensor was characterized by electrochemical impedance spectroscopy (EIS). The electrochemical characteristics of the biosensor were studied by cyclic voltammetry and chronoamperometry. Experimental conditions were investigated which influence the performance of the biosensor, such as pH, and applied potential. The biosensor showed a linear response to H_2O_2 over a concentration range from 7.0×10^{-6} to 4.1×10^{-3} M with a detection limit of 1.8×10^{-6} M based on a signal-to-noise ratio of 3 under optimum conditions. The K_M^{app} value of HRP in the composite was evaluated to be 1.38 mM. The biosensor obtained from this study possesses high sensitivity, good reproducibility, and long-term stability.

1. Introduction

Amperometric biosensors have attracted considerable interest in the field of clinical, biomedical analysis, chemical and pharmaceutical laboratories [1–5]. However, the exciting possibility of biosensors is usually hindered by their poor stability and short shelf life. To improve the stability and extend the shelf life of biosensor, various techniques such as cross-linking [6], sol–gel/hydrogel [7], self-assembly [8], covalent binding [9], and surfactant–enzyme complex [10,11] have been explored. Recently, nanomaterial such as carbon nanotubes [12,13], zirconium oxide [14], and nanometer-sized gold colloid particles [15] are also used for the fabrication of composite electrodes. The electro-co-deposition technology, as an effective immobilization of enzyme within a biocompatible material by using simple and controllable procedure, is of great significance and still being practiced today [16,17]. The advantages of the strategy come from the following two aspects. Firstly, electrochemical deposition process offers a simple and convenient technique for the fabrication of enzyme electrodes with minimum denaturation and strong adherence to electrode surface compared with other procedures [18,19]. Secondly, the biosensor fabrication is reproducible and the thickness of the resulting biocomposite film is controllable. However, the electro-enzymatic activity of the immobilized enzyme depends on the chemical and physical properties of matrix employed in the immobilization process. And the leakage of enzyme from matrix in the testing surrounding is generally recognized. Consequently, more innovative approaches for enzyme immobilization have to be sought particularly for the applications in biomedical, biocatalytic, and biosensing fields [18,20].

Alginate is, being an anionic polymer with carboxyl groups, a natural and biocompatible polymer which can provide microenvironments to improve the enzyme stability or maintain its bioactivity [21–33]. Due to the possessing of carboxyl groups, alginate is a pH shift polymer. As a result, the solubility and net charge of alginate is pH-dependent. The deposition is performed based on the pH decrease at the anode owing to the decomposition of water. The electro-co-deposition film posed uniform coatings on substrates of complex shapes [34]. But no report on immobilization of biomolecules such as HRP by electro-co-deposition with alginate was found, to the best of our knowledge, in the literature.

It is worth emphasizing the two novel features in this study: First, natural biocompatible polymeric alginate was utilized as a matrix; second, the employment of electro-co-deposition technique during fabrication. In this paper, we attempt to disclose implementation of the above mentioned strategies for the successful development of an electrochemical biosensor which displays relatively high sensitivity, acceptable stability and good repeatability. The preparation method and main characteristic features were described and discussed in detail.

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2. Experimental

2.1. Reagent and materials

Horseradish peroxidase (HRP, EC 1.11.1.7, 250 U mg^{-1}) was obtained from Sigma. Sodium alginate (SA) was purchased from Shanghai Guoyao Chemical Co. Ltd., China. Polyvinyl butyral (PVB, 98%) was bought from Shanghai Chemical Reagent Co. Ltd., China. 0.1 M phosphate buffers solution (PBS) containing 0.1 M KCl with various pH values were prepared by mixing the stock solutions of K_2HPO_4 and KH_2PO_4, and adjusted by 0.1 M KOH and 0.1 M H_3PO_4 solution. Hydrogen peroxide (30%, w/v solution) was purchased from Chemical Reagent Company, Chongqing, China, and the solutions of the more diluted hydrogen peroxide were prepared freshly using a 30% hydrogen peroxide and determined by titration with potassium permanganate. All reagents were of analytical reagent grade and used as received. Double distilled water was used in this experiment throughout.

2.2. Electrode modification procedure

A bulk gold disk electrode (d = 4 mm) was carefully polished with 1.0, 0.3 and 0.05 μm alumina slurry, and ultrasonically cleaned in ethanol and double distilled water before modification. The gold electrode was then chemically cleaned by repeatedly scanning, at the potential range of −0.3 to 1.5 V, in a 0.5 M H_2SO_4 solution until the voltammograms were reproducible. HRP–SA/Au electrode was achieved by cycling at the potential between −1.0 and 1.0 V for 25 consecutive cycles in 5 ml sodium alginate (2%, w/v) doped with 2 mg/ml HRP (PBS, pH 6.0). Then, the HRP–SA/Au electrode was dipped into PVB ethanol solution (2%, w/v) to a depth of 10 mm for 10 min.

2.3. Apparatus

Cyclic voltammetry (CV) and chronoamperometry (CA) were carried out on AUTOLAB system with PGSTAT12 (Eco Chemie B.V., Utrecht, Netherlands). The electrochemical cell consists of a three-electrode system, bare or modified gold electrodes were employed as working electrode, platinum wire as auxiliary electrode and saturated calomel as reference electrode (SCE). Electrochemical impedance spectroscopy (EIS) was performed with a Model IM6e (ZAHNER Elektrik, Germany). UV–vis absorption spectra were recorded in the range of 250–800 nm using a Lambda 17 UV–vis 8500 spectrometer (PE Co, USA) with a quartz cell (path length 1 cm). All potentials were measured and reported versus the SCE and all experiments were carried out at room temperature.

3. Results and discussion

3.1. Fabrication of the H_2O_2 biosensor

The possibility of deposition of alginate and composite coatings for surface modification of materials opens new opportunities in the fabrication of advanced biomedical implants [34]. In the work, an electrochemical sensing platform was developed based on the integration of natural polysaccharide alginate and enzyme (HRP). Alginate is an anionic polymer with carboxyl groups and ideally suited for electrodeposition since its net charge and solubility are pH dependent. Electro-co-deposition was used for one-step construction of H_2O_2 biosensors by local formation of SA–HRP biocomposite film on the surface of electrode (Fig. 1). The results of deposition of alginate doped HRP suggested that the alginate composite film with HRP were successfully fabricated on the surface of gold electrode. The proposed mechanism of gel formation is

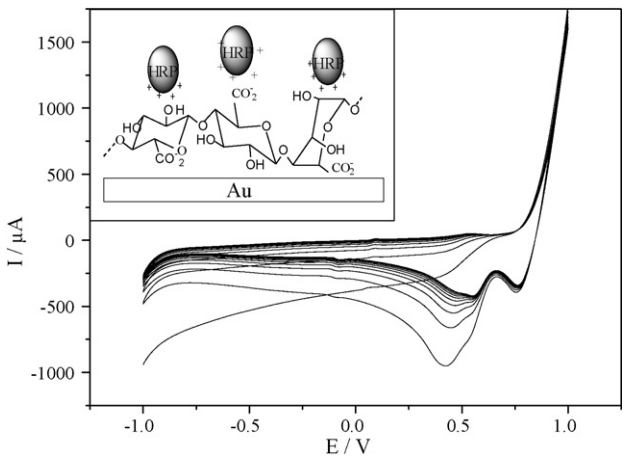

Fig. 1. The cyclic voltammograms of HRP–SA composites progressive course on bare Au electrode between −1.0 and 1.0 V (vs. SCE) at a scan rate of 100 mV/s for 25 consecutive scans. Insert: illustration of the electro-co-deposition process of modified electrode.

based on the pH decrease at the anode owing to the electrochemical decomposition of water [35].

$$H_2O \rightarrow \frac{1}{2}O_2 + 2H^- + 2e^- \quad (1)$$

The dissociation of sodium alginate (Na–SA) results in the formation of anionic SA^- species.

$$Na\text{–}SA \rightarrow Na^+ + SA^- \quad (2)$$

It is suggested that SA^- species formed alginic acid (H–SA) gel in the low pH region around the electrode.

$$SA^- + H^+ \rightarrow H\text{–}SA \quad (3)$$

Obtained bulk gel film incorporated with HRP is locally electro-co-deposited on the anode surface (gold electrode). Then, the coating of PVB was to prevent the leakage of HRP out of HRP–SA composite. After storing for about 24 h at 4 °C, the PVB/HRP–SA/Au electrode was ready for testing.

3.2. UV studies of the interaction between HRP and SA

UV–vis absorbance spectroscopy was employed to characterize the conformational change of protein and the interaction between protein and other compositions [36]. UV–vis spectra of SA, HRP and HRP–SA composites were presented in Fig. 2. The adsorption peak of HRP is 403 nm (Fig. 2b), while no shift is observed when HRP is immobilized in the HRP–SA composites (Fig. 2c). After stored in a refrigerator at 4 °C for 7 days, the absorption peak of HRP–SA composites still remain at 403 nm (Fig. 2 c2), but shifted to 405 nm (Fig. 2 c3) 1 month later. This indicates that the interaction between HRP and SA does not destroy the conformational structure of HRP. Instead, SA may provide a microenvironment for HRP to maintain its native structure and bioactivity as reported by others [31,32].

3.3. Electrochemical impedance spectroscopy (EIS) of the modifying process

EIS is one of the most effective electro-analytical techniques used in studying the interfacial properties of modified electrodes. EIS is particularly useful for understanding the chemical transformations and processes associated with the conductive supports [37,38]. The typical impedance spectrum (presented in the form of the Nyquist plot) includes a semicircle portion at higher frequencies corresponding to the electron-transfer-limited process and a linear

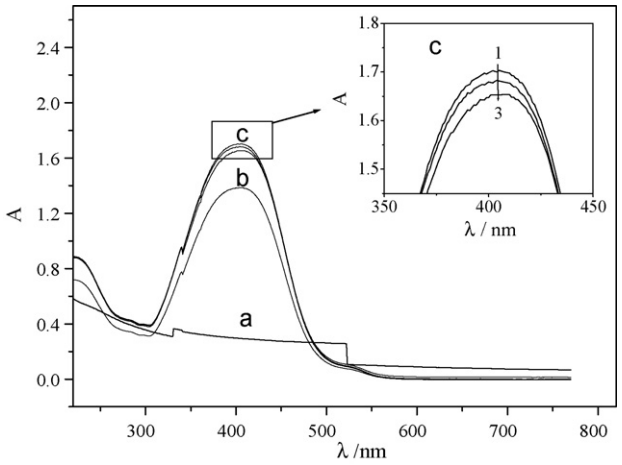

Fig. 2. UV–vis absorption spectra of SA (a), HRP (b), and HRP–SA (c) in 0.1 M PBS (pH 6.0).

part at lower frequency range representing the diffusion limited process. The semicircle diameter in the impedance spectrum equals the electron-transfer resistance, R_{et}. Such resistance controls the electron-transfer kinetics of the redox probe at the electrode interface. Fig. 3 shows the results of Faradic impedance spectroscopy of a bare gold electrode (Fig. 3a), SA/Au electrode (Fig. 3b), HRP–SA/Au electrode (Fig. 3c) and PVB/HRP–SA/Au electrode (Fig. 3d) in 5 mM $K_4Fe(CN)_6/K_3Fe(CN)_6$ (1:1) solution containing 0.1 M KCl. It is observed that the bare gold electrode exhibits no semicircle but an almost straight line, which implies the characteristic of a diffusion limiting step of the electrochemical process. After being modified with SA composite, the semicircle increases, indicating that SA film as barriers make interfacial charge transfer more difficult. When HRP–SA biocomposite is fabricated onto Au electrode surface by electro-co-deposition, the R_{et} of HRP–SA/Au electrode increases compared with that of the SA/Au electrode due to the increase in the quantity of HRP assembled, which signify that HRP is successfully immobilized onto the electrode. Finally, after being modified with PVB, the EIS of HRP–SA/Au electrode shows a higher interfacial resistance (R_{et}), indicating that PVB obstructs electron-transfer of the electrochemical probe. On the basis of the EIS results, we can conclude that HRP has been successfully immobilized on the gold electrode with SA and PVB as matrices.

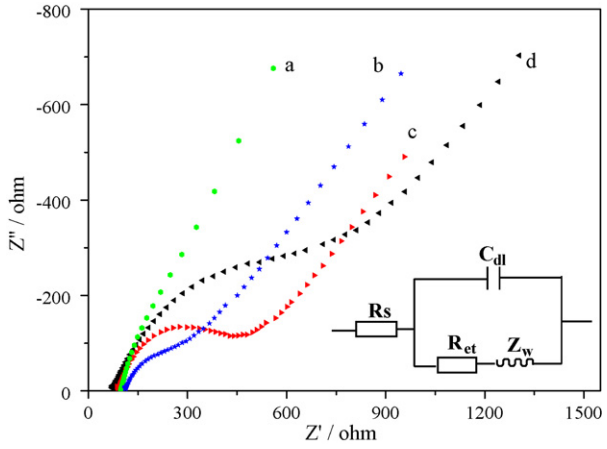

Fig. 3. EIS of (a) bare gold electrode, (b) SA/Au electrode, (c) HRP–SA/Au electrode, and (d) PVB/HRP–SA/Au electrode in a background solution of 5 mM $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ (1:1) PBS (0.1 M, pH 6.0).

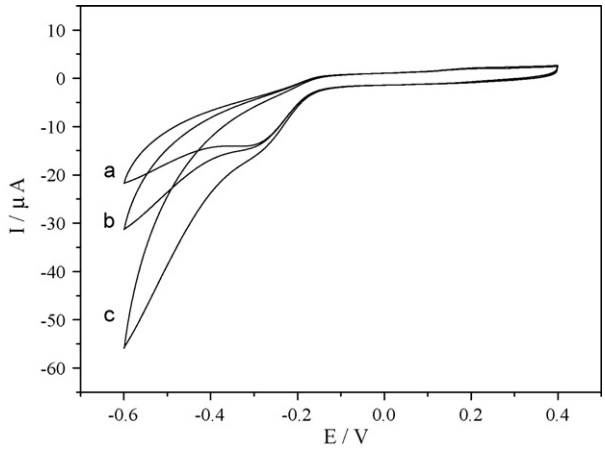

Fig. 4. CVs of the biosensor at 0.1 M PBS (pH 6.0) in the absence (a), presence of 0.05 mM H_2O_2 (b) and 0.15 mM H_2O_2 (c). Scan rate: 50 mV/s.

3.4. Electrochemical response to hydrogen peroxide at the biosensor

The electrocatalytic reactivity of the enzyme electrode towards H_2O_2 was investigated by cyclic voltammetry (CA). Fig. 4 shows the catalytic reduction of H_2O_2 in the biosensor at 0.1 M PBS of pH 6.0. In the absence of H_2O_2, no obvious current is found (Fig. 4a), but an obviously catalytic current is detected in the presence of 0.05 mM H_2O_2 (Fig. 4b) and 0.15 mM H_2O_2 (Fig. 4c). The results above indicate HRP–SA composite shows good catalytic activity toward hydrogen peroxide. The reaction mechanism of the H_2O_2 biosensor was briefly summarized as below: In the presence of H_2O_2, HRP (Fe^{2+}) is efficiently converted to its oxidized form, HRP (Fe^{3+}). HRP (Fe^{3+}) then reduced at the electrode surface according to direct electron transfer [39].

3.5. Influence of pH and applied potential on biosensor response

In order to obtain an efficient biosensor for H_2O_2, the influences of pH and applied potential on response of PVB/HRP–SA/Au electrode were investigated. The change of chronoamperometric current with pHs range from 4.0 to 9.0 under constant hydrogen peroxide concentration (0.2 mM) was shown in Fig. 5A. Apparently, with an increase of pH value, the peak current of the PVB/HRP–SA/Au electrode increases and then decreases. The maximum response appears at pH 6.0. Therefore, the buffer solution of pH 6.0 was selected for further experiments.

Fig. 5B shows the dependence of the chronoamperometric current response to constant hydrogen peroxide concentration (0.2 mM) on the applied potential in the range of −0.6 to 0 V. Amperometric current increases gradually when the applied potential shifts from 0 to −0.6 V. Taking the sensitivity and selectivity into consideration, the applied potential of −0.3 V was chosen for the amperometric determination of H_2O_2 in this work.

3.6. Amperometric detection of hydrogen peroxide biosensor

The amperometric responses to H_2O_2 of variously modified electrodes were investigated by successively adding H_2O_2 to a continuously stirred PBS solution of pH 6.0, applied potential of −0.3 V. Fig. 6 shows typical current–time curves of variously modified electrodes for successive addition of H_2O_2 of different concentration. As can be seen, for the bare electrode, the response current is inconspicuous with the addition of H_2O_2 (Fig. 6a), and the current response of the PVB/SA/Au electrode is decreased by a little

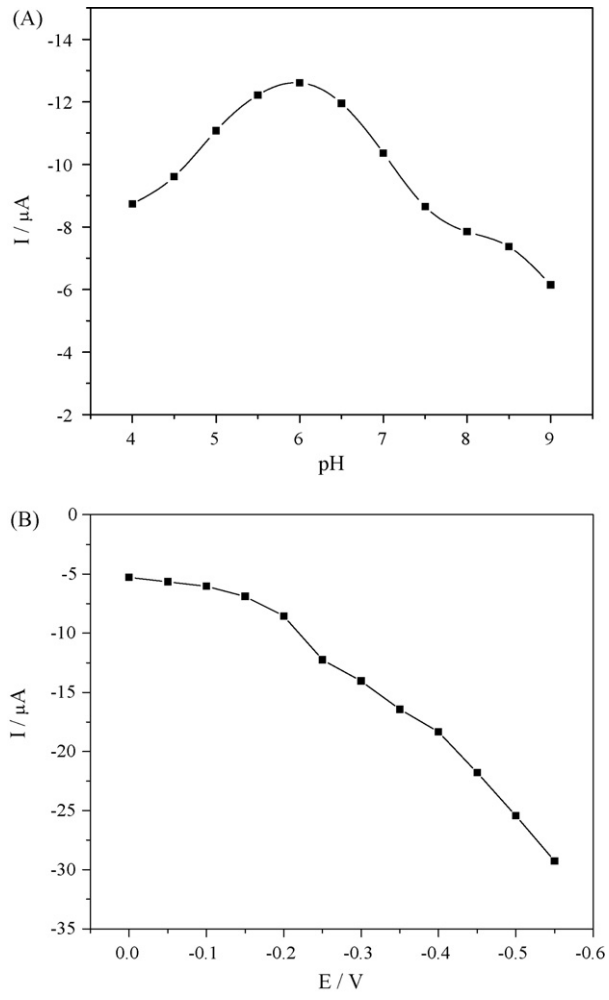

Fig. 5. Dependence of the current response of the biosensor to 0.2 mM H_2O_2 on the pH of buffer solutions at an applied potential of −0.3 V (A) and on the applied potential in 0.1 M PBS (pH 6.0) (B).

bit (Fig. 6b), the reason is that both PVB and SA may hamper the transfer of electrons. As comparison, the PVB/HRP–SA/Au electrode shows a larger response to H_2O_2 (Fig. 6c), which indicates that SA contributes an effective effect to the immobilization of HRP. The

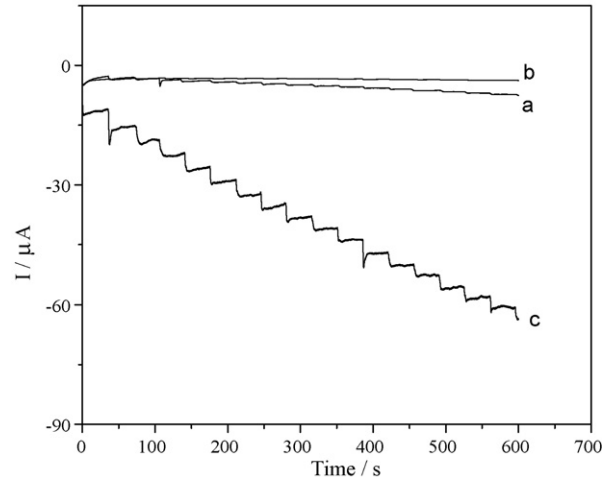

Fig. 6. The chronoamperometric response curve for successive addition of 0.35 mM H_2O_2 obtained by bare Au electrode (a), PVB/SA/Au electrode (b) and PVB/HRP–SA/Au electrode (c) at applied potential of −0.3 V, in 0.1 M PBS (pH 6.0).

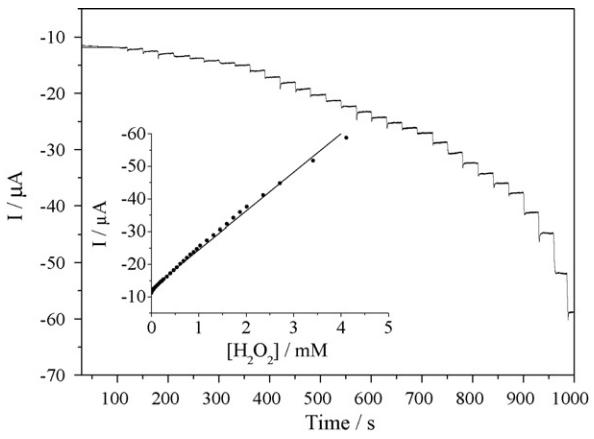

Fig. 7. Amperometric response of the biosensor to H_2O_2 in 0.1 M PBS (pH 6.0) at an applied potential of −0.3 V upon successive additions of different concentration in the time intervals of 30 s. Insert: linear calibration curves.

result of larger response might attribute to high activity of enzyme HRP in SA composite matrices.

The chronoamperometric response of the hydrogen peroxide biosensor was investigated by successively adding H_2O_2 to a continuously stirred PBS solution under optimum conditions. The response current increases with increasing concentration of H_2O_2 as illustrated in Fig. 7. We used the steady-state current to plot with the concentration of H_2O_2. There is a linear relation of the current with concentration of H_2O_2 ranging from 7.0×10^{-6} to 4.1×10^{-3} M, as shown in the insert of Fig. 7. The linear regression equation is $i(\mu A) = -12.51 - 11.91 c_{H_2O_2}$ (mM) with a correlation coefficient of 0.9974 ($n = 33$). From the slope of calibration curve, the detection limit of 1.8×10^{-6} M is estimated at signal-to-noise ration of 3. In addition, the proposed biosensor achieves 95% of the steady-state current in less than 5 s. Such rapid response could attribute to the direct electrical communication between HRP in the composite and the electrode.

The apparent Michaelis–Menten constant (K_M^{app}), which gives an indication of the enzyme-substrate kinetics, can be obtained from the electrochemical version of the Lineweaver–Burk equation [40]:

$$\frac{1}{I_{ss}} = \frac{1}{I_{max}} + \frac{K_M^{app}}{I_{max} C}$$

where I_{ss} is the steady-state current after the addition of substrate, which can be obtained from amperometric experiments, I_{max} the maximum current under saturated substrate condition and C is the bulk concentration of the substrate. K_M^{app} is the apparent Michaelis–Menten constant and a characteristic parameter of the enzyme- substrate kinetics. Accordingly, the value of K_M^{app} is estimated to be 1.38 mM. The K_M^{app} value of PVB/HRP–SA/Au (1.38 mM) was higher than that of free horseradish peroxidase (0.152 mM) [41]. The higher K_M^{app} value for the immobilized enzymes may be a result of a number of effects. The migration of substrate from the solution to the microenvironment of an immobilized enzyme can be a major factor. The substrate concentration on diffusion film is lower than in the solution. The rate at which substrate passes over the diffusion film, which in turn determines the concentration of substrate in the vicinity of the enzyme and hence the K_M^{app} values [42]. The apparent Michaelis–Menten constant (K_M^{app}) of PVB/HRP–SA/Au developed in this work is close to 1.1 mM for HRP/Au nanoparticles/cysteine-silica sol–gel modified gold electrode [43], 1.3 mM for HRP/AuNP/SPCE [44], 1.22 mM for HRP–AuNPs–SF/GCE [45]. This value of K_M^{app} indicates that HRP molecules immobilized on SA composites retain their bioactivity

Table 1
Possible interferences tested with the hydrogen peroxide biosensor.

Possible interferences	Current ratio[a]	R.S.D.
Glucose	0.93 ± 0.08	3.2
Fructose	0.92 ± 0.05	2.9
L-Cysteine	0.99 ± 0.12	3.8
L-Leucine	1.02 ± 0.11	3.4
L-Tyrosine	0.98 ± 0.09	3.6
Ascorbic acid	1.05 ± 0.14	4.1
Uric acid	1.02 ± 0.10	3.6

[a] Each value (mean of three measurements) was obtained by comparing the current for a mixture of a 0.20 mM interfering substance and 0.10 mM H_2O_2 with that for 0.10 mM H_2O_2 alone. Assay solution: 0.10 M PBS (pH 6.0). Applied potential applied: −0.3 V.

and exhibit high affinity for H_2O_2. However, the K_M^{app} value for PVB/HRP–SA/Au is markedly higher than that obtained at HRP/Au colloid/cysteamine/glutaraldehyde/cysteamine modified gold electrode (0.094 mM) [46], which indicated that HRP immobilized on SA may partially adsorb on bulk gold electrode surface and decrease its affinity for H_2O_2 [45].

3.7. Repeatability, reproducibility and stability of the hydrogen peroxide biosensor

A relative standard deviation (R.S.D.) of 3.7% was acquired for twenty successive measurements at 0.1 mM H_2O_2. The electrode-to-electrode reproducibility was estimated by determining the amperometric responses to H_2O_2 concentration in duplicating with each of four different H_2O_2 biosensors of the same type independently. The coefficient was calculated to be 4.2%, showing fabrication reproducibility acceptable (see supplementary material). The good reproducibility can be ascribed to the non-manual approach with simplicity, controllability and reproducibility. Furthermore, the storage stability of the proposed biosensor was also studied. The biosensor was immersed in 0.1 M PBS (pH 6.0) at 4 °C when not used. The biosensor decreased 3.5%, 8.7%, and 16% of the initial response after 3 days, 2 weeks, and 1 month storage, respectively. The good storage stability was ascribed to the good biocompatibility of the SA–HRP biocomposite, which can provide a favorable microenvironment for the entrapped enzyme. The noticeable retention of electrocatalytic activity of the immobilized enzyme may be caused by the formation of poly-electrostatic interactions between the positive charged enzyme and the negative charged alginate. In addition, the coating of PVB should avoid the release of the enzyme from the electrode surface, maintaining the stable response signals after several days.

3.8. Selectivity and recovery experiments of the developed H_2O_2 biosensor

In our experiment, seven interfering substances (glucose, fructose, L-cysteine, L-leucine, L-tyrosine, ascorbic acid, uric acid) were used to examine the selectivity of the biosensor. The current ratios were calculated by reading the current of the biosensor in the assay solution containing 0.1 mM H_2O_2 and a 0.2 mM interfering substance, comparing it with the current from the proposed biosensor in the same assay solution containing only 0.1 mM H_2O_2. The results are listed in Table 1. It can be observed that seven tested interferents could not significantly interfere with the determination of H_2O_2, which is largely attributed to the low working potential of −0.3 V used in the determination of H_2O_2.

To demonstrate the analytical applicability of the biosensor, the recoveries of two H_2O_2 samples were determined by the standard adding method. The results were satisfactory. As listed in Table 2, the recovery rate was in the range 95.2–102.4%.

Table 2
Recovery studies of the biosensor for determining H_2O_2.

$C_{original}$ (mM)	C_{added} (mM)	C_{found} (mM)	Recovery (%)*
0.250	0.001	0.257	102.4
	0.250	0.492	98.4
0.750	0.020	0.754	97.9
	0.500	1.190	95.2

* Recovery (%) = $C_{found}/(C_{original} + C_{added})$.

4. Conclusion

A novel and simple hydrogen peroxide biosensor based on electro-co-deposition of sodium alginate and horseradish peroxidase was investigated in this study. The biosensor showed a linear response to H_2O_2 over a concentration range from 7.0×10^{-6} to 4.1×10^{-3} M with a detection limit of 1.8×10^{-6} M based on a signal-to-noise ratio of 3 under optimum conditions. The K_M^{app} value of HRP in the composite was evaluated to be 1.38 mM. The advantages of this biosensor are: (i) easy immobilization procedure, (ii) good electrocatalytical response to the reduction of H_2O_2; (iii) high selectivity and stability.

Acknowledgements

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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.molcatb.2009.04.015.

References

[1] S. Brahim, D. Narinesingh, A. Guiseppi-Elie, Anal. Chim. Acta 448 (2001) 27–36.
[2] H. Nakamura, I. Karube, Anal. Bioanal. Chem. 377 (2003) 446–468.
[3] L.A. Terry, S.F. White, L.J. Tigwell, J. Agric. Food Chem. 53 (2005) 1309–1316.
[4] D. Shan, M.J. Zhu, H.G. Xue, S. Cosnier, Biosens. Bioelectron. 22 (2007) 1612–1617.
[5] C. Camacho, J.C. Matı´as, D. Garcı´a, B.K. Simpson, R. Villalonga, Electrochem. Commun. 9 (2007) 1655–1660.
[6] L. Qian, X. Yang, Talanta 68 (2006) 721–727.
[7] B.Q. Wang, J.Z. Zhang, G.J. Cheng, S.J. Dong, Anal. Chim. Acta 407 (2000) 111–118.
[8] G. Wang, H. Lu, N. Hu, J. Electroanal. Chem. 599 (2007) 91–99.
[9] L. Doretti, D. Ferrara, S. Lora, G. Palma, Biotechnol. Appl. Biochem. 29 (1999) 67–72.
[10] S. Mahiuddin, A. Renoncourt, P. Bauduin, D. Touraud, W. Kunz, Langmuir 21 (2005) 5259–5262.
[11] Y.M. Yan, W. Zheng, M.N. Zhang, L. Wang, L. Su, L.Q. Mao, Langmuir 21 (2005) 6560–6566.
[12] M.Y. Yang, Y. Yang, H.F. Yang, G.L. Shen, R.Q. Yu, Biomaterials 27 (2006) 246–255.
[13] J. Wang, M. Li, Z. Shi, N. Li, Z. Gu, Anal. Chem. 74 (2002) 1993–1997.
[14] G. Zhao, J.J. Feng, J.J. Xu, H.Y. Chen, Electrochem. Commun. 7 (2005) 724–729.
[15] H.Y. Gu, A.M. Yu, H.Y. Chen, J. Electroanal. Chem. 516 (2001) 119–126.
[16] R. Garjonyte, A. Malinauskas, Sens. Actuators B 56 (1999) 85–92.
[17] A. Mittal, S. Khurana, H. Singh, R.C. Kamboj, Enzyme Microb. Technol. 3 (2005) 318–323.
[18] Y. Itoh, M. Matsusaki, T. Kida, M. Akashi, Biomacromolecules 7 (2006) 2715–2718.
[19] Z.Q. Tong, R. Yuan, Y.Q. Chai, Y. Xie, S.H. Chen, J. Biochem. 128 (2007) 567–575.
[20] S.W. Xu, Y. Lu, Z.Y. Jiang, H. Wu, J. Mol. Catal. B: Enzym. 1–4 (2006) 68–73.
[21] F.A. Johnson, D.Q.M. Craig, A.D. Mercer, J. Pharm. Pharmacol. 49 (1997) 639–643.
[22] G. Orive, R.M. Hermandez, A.R. Gascon, R. Calafiore, T.M. Chang, P. De Vos, G. Hortelano, D. Hunkeler, I. Lacik, A.M. Shapiro, J.L. Pedraz, Nat. Med. 9 (2003) 1104–1106.
[23] N. Munjal, S.K. Sawhney, Enzyme Microb. Technol. 30 (2002) 613–619.
[24] R.E. Ionescu, K. Abu-Rabeah, S. Cosnier, R.S. Marks, Electrochem. Commun. 7 (2005) 1277–1282.
[25] S. Cosnier, A. Novoa, C. Mousty, R.S. Marks, Anal. Chim. Acta 453 (2002) 71–79.
[26] C.M. Silva, A.J. Ribeiro, I.V. Figueiredo, A.R. Goncalves, F. Veiga, Int. J. Pharm. 311 (2006) 1–10.
[27] F. Gu, B. Amsden, R. Neufeld, J. Control. Release 96 (2004) 463–472.
[28] I. Freeman, A. Kedem, S. Cohen, Biomaterials 29 (2008) 3260–3268.

[29] W. Sun, D. Wang, R.F. Gao, K. Jiao, Electrochem. Commun. 9 (2007) 1159–1164.
[30] C.F. Ding, M.L. Zhang, F. Zhao, S.S. Zhang, Anal. Biochem. 78 (2008) 32–37.
[31] R.E. Ionescu, K. Abu-Rabeah, S. Cosnier, C. Durrieu, J.M. Chovelon, R.S. Marks, Electroanalysis 18 (2006) 1041–1051.
[32] S. Cosnier, C. Mousty, J. de Melo, A. Lepelle, A. Novoa, B. Polyak, R.S. Marks, Electroanalysis 16 (2004) 2022–2029.
[33] P. Trens, R. Valentin, F. Quignard, Colloids Surf. A: Physicochem. Eng. Aspects 296 (2007) 230–237.
[34] M. Cheong, I. Zhitomirsky, Colloids Surf. A: Physicochem. Eng. Aspects 328 (2008) 73–78.
[35] F. Yokoyama, T. Fujino, K. Kimura, Y. Yamashita, K. Nagata, H. Mihashi, Eur. Polym. J. 34 (1998) 229.
[36] Y.C. Zhu, G.J. Cheng, S.J. Dong, Biophys. Chem. 97 (2002) 129–138.
[37] C.R. Foschini, D.P.F. Souza, P.I. Paulin Filho, J.A. Varela, J. Eur. Ceram. Soc. 21 (2001) 1143–1150.
[38] F. Sundfors, J. Bobacka, A. Ivaska, A. Lewenstam, Electrochim. Acta 47 (2002) 2245–2251.
[39] G. Wang, J. Xu, H. Chen, Z. Lu, Biosens. Bioelectron. 18 (2003) 335–343.
[40] R.A. Kamin, G.S. Wilson, Anal. Chem. 5 (1980) 1198–1205.
[41] X.M. Jiang, Y.M. Li, S.M. Yang, Z.C. Chen, X.F. Lin, J. Chin. Instrum. Anal. 23 (2004) 50–52.
[42] S. özlem Alptekin, S. Seyhan Tükel, D. Yıldırım, D. Alagöz, J. Mol. Catal. B: Enzym. 58 (2009) 124–131.
[43] J. Di, C. Shen, S. Peng, Y. Tu, S. Li, Anal. Chim. Acta 553 (2005) 196–200.
[44] X. Xu, S. Liu, H. Ju, Sensors 3 (2003) 350–360.
[45] H. Yin, S. Ai, W. Shi, L. Zhu, Sens. Actuators B 137 (2009) 747–753.
[46] Y. Xiao, H. Ju, H. Chen, Anal. Chim. Acta 391 (1999) 73–82.