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Short communication

Sensitive amperometric biosensor for phenolic compounds based on graphene–silk peptide/tyrosinase composite nanointerface

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ABSTRACT

New graphene–silk peptide (Gr–SP) nanosheets were prepared and successfully fabricated with tyrosinase (Tyr) as a novel biosensor for the determination of phenolic compounds. The Gr–SP nanosheets were fully characterized with transmission electron microscopy, x-ray diffraction, x-ray photoelectron spectroscopy, UV/Vis and FTIR spectra. The developed biosensors were also characterized with scanning electronic microscopy and electrochemical impedance spectroscopy. Using bisphenol A (BPA) as a model substrate in the sensing system, a number of key factors including the volume of Gr–SP–Tyr solution, the applied potential, pH values, temperature, and the Tyr/Gr–SP ratio that influence the analytical performance of the biosensor were investigated. The biosensor gave a linear response on the concentration ranges of 0.001–16.91 μM for catechol with the sensitivity of 7634 mA M^{-1} cm^{-2}, 0.0015–21.12 μM for phenol with the sensitivity of 4082 mA M^{-1} cm^{-2}, and 0.002–5.48 μM for BPA with the sensitivity of 2511 mA M^{-1} cm^{-2}. The low detection limits were estimated to be 0.23, 0.35 and 0.72 nM (S/N=3) for catechol, phenol and BPA, respectively. The biosensors also exhibit good repeatability and long-term stability. The practical application of the biosensor was also demonstrated by the determination of BPA leaching from commercial plastic drinking bottles.

1. Introduction

Graphene has emerged as an attractive nanomaterial since it was discovered by Geim and co-workers (Novoselov et al., (2004). It is proved that graphene possesses unique structural features and extraordinary chemical, electrical, and mechanical properties (Novoselov et al., 2007), and it is widely used in the fabrication of field-effect transistors (Standley et al., 2012), optoelectronic devices (Chung et al., 2010), supercapacitors (Huang et al., 2012), pH sensor (Ang et al., 2008), chemical sensors (Liu et al., 2012; Sheng et al., 2012) and biosensors (Liu et al., 2011; Pumera, 2011; Wang et al., 2012; Zhu et al., 2012). Most of its remarkable performances are based on individual sheet (Shan et al., 2009). In order to prevent from aggregating, many efforts have been devoted to functionalize graphene through both covalent (Shan et al., 2009), non-covalent interactions (Xu et al., 2008) and polymeric modifications (Qi et al., 2010). Nowadays, the utility of the monolayer graphene is largely expanded.

Biomolecules, such as DNA, enzymes and proteins, can also be non-covalently adsorbed onto a graphene surface to create a lot of graphene-based nanostructures consisting of bio-recognition units (Luo et al., 2012). In addition, the covalent modification of a graphene oxides (GO) surface is also considered to be a useful method. In the process, the epoxy groups on the GO surface are the reactive sites for the nucleophilic substitution reaction with amine ($-NH_2$) functionality of the organic modifiers. Aliphatic and aromatic amines, amino acids, ionic liquids, small molecular weight polymers and amine terminated biomolecules have been successfully used in the preparation of the functionalized graphene (Laaksonen et al., 2010; Yang et al., 2009; Shan et al., 2009).

In this study, we reported the first use of silk peptide (a partly hydrolysis product of silk protein) as a modifier to functionalize graphene to produce new graphene–silk peptide composite nanosheets. The obtained Gr–SP nanosheets possess high conductivity and biocompatibility and are able to provide a superior nanointerface to immobilize biomaterials for biosensors fabrication. Tyrosinase was fabricated with these Gr–SP nanosheets to use as a biosensor for the detection of various phenolic compounds such as catechol, phenol and bisphenol A. Because of the high toxicity of these phenolic substances, the development of a

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reliable analytical methodology for identifications and quantifications for the compounds is of great importance for environment and human health protection.

Electrochemical biosensor is considered as one of the best choices for in situ monitoring of phenolic compounds (Wang et al., 2008). The biosensor is usually developed based on monitoring the reduction signal of the quinine species, which is generated by the catalysis of tyrosinase in the presence of molecular oxygen. Therefore, effective immobilization of tyrosinase onto an electrode surface is a key step in the development of tyrosinase biosensors. In this study, Gr–SP nanosheets were demonstrated to be the excellent biocompatible nanointerface to maintain good catalytic activity of tyrosinase and provide good sensitivity towards phenolic compounds.

2. Experimental

The details of chemicals, apparatus and analytical data for the characterization of Gr–SP nanosheets (from Figs. S1–S5) can be found in the Supporting information section.

2.1. Synthesis of Gr–SP nanosheets

Amounts of 4.0 mg graphene oxides, 20.0 mg of SP and 16.0 mg of KOH were added into 20.0 mL of ultrapure water, and were vigorously stirred in order to obtain a homogeneous mixture. The obtained mixture was kept stirring at 80 °C for 24 h. After that, 2.0 mL $NaBH_4$ solution (1.0 M) was added to reduce graphene oxides. The reaction was kept at 80 °C for 2 h. The synthesized graphene–SP nanosheets were collected by centrifugation, and were thoroughly washed with water to remove the impurities and the excess of SP. The yield estimated on the basis of the amount of materials recovered and the added for reaction was 40.8% approximately.

2.2. Fabrication of Gr–SP–Tyr biosensor

Glassy carbon electrode (GCE) was polished with emery paper and alumina slurries followed by rinsing thoroughly with ultrapure water. The electrodes were cleaned by successively ultrasonication in nitric acid, ethanol and water, and then allowed to dry at room temperature. A solution of 8.0 mg mL^{-1} of tyrosinase was prepared with 1/15 M phosphate buffer solution (PBS) at pH 7.4. Gr–SP nanosheets were dispersed into a phosphate buffer solution with ultrasonication to give a homogeneously solution (1.0 mg mL^{-1}). After that, 100.0 μL tyrosinase solution (400 units) and 100.0 μL of the Gr–SP solution were thoroughly mixed. A volume of 5.0 μL mixed solution was drop coated onto the cleared glassy carbon electrode surface, and then was dried at 4 °C to obtain a film. The Gr–SP–Tyr film modified electrode was treated with glutaraldehyde vapor for 5 min for the preparation of Gr–SP–Tyr biosensor. After that, the biosensor was fully washed with ultrapure water to remove the physically absorbed chemicals.

2.3. Samples preparation

Plastic drinking bottles were bought from a local supermarket, and were cut into small pieces in a diameter of 1 cm. The pieces of plastic drinking bottles were refluxed in ethanol for 5 h, and were kept into ethanol for 24 h under room temperature. The mixture was filtered with 0.45 μm membrane. After complete evaporation of the solvent, 5.00 mL ethanol was added to the residue, then diluted to 25.00 mL with PBS and used for BPA assay.

2.4. Electrochemical measurements

Cyclic voltammetric measurements were performed in a phosphate buffer solution (pH 6.0) which was saturated with oxygen. Cyclic voltammograms of 0.1 mM BPA were recorded in the potential range from −0.40 to 0.40 V. Amperometric determination of phenolic compound was carried out with an applied potential of −0.10 V in an electrochemical cell containing 25.00 mL of phosphate buffer solution saturated with oxygen. When a steady background signal was observed, phenolic compounds were successively injected into the solution under stirring conditions, and the amperometric response curves were recorded accordingly.

3. Results and discussion

3.1. Scanning electronic microscopy characterization

SEM images of the Gr–SP/GCE (a) and the Gr–SP–Tyr/GCE (b) were depicted in Fig. 1. It was found that the Gr–SP/GCE displayed tiny wrinkles over the whole surface with mountainous peaks. The overlap of Gr–SP nanosheets with two or more layers was observed obviously. Moreover, some worms-like rugby balls were also found clearly spread onto the Gr–SP–Tyr film electrode surface. These worms are tyrosinases which were immobilized on the biocompatible Gr–SP nanointerface through the covalent cross-linking of amine group in SP and Tyr with glutaraldehyde. The successful immobilization of Tyr on the Gr–SP nanointerface was also confirmed by electrochemical impedance spectroscopy (Fig. S6).

3.2. Cyclic voltammetric behaviors of BPA

Bisphenol A was employed as a model substrate to investigate the electrochemical behavior of phenolic compounds at the Gr–SP–Tyr biosensor. Cyclic voltammograms of 0.1 mM BPA at the Gr–SP–Tyr/GCE (a), Tyr/GCE (b), Gr–SP/GCE (c) and the unmodified GCE (d) in an oxygen saturated PBS were depicted in Fig. 2. A

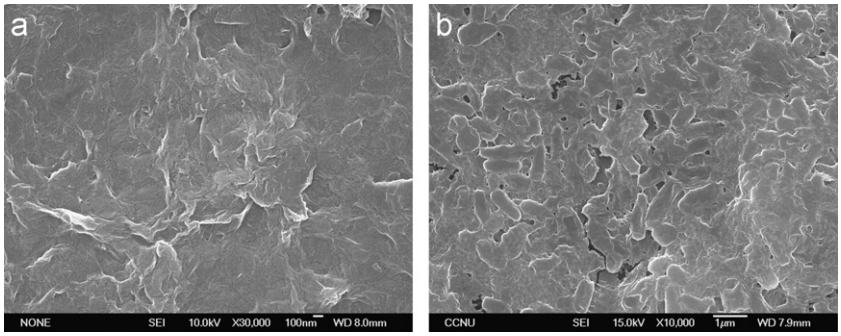

Fig. 1. SEM images of Gr–SP/GCE (a) and Gr–SP–Tyr/GCE (b).

couple of well-defined redox peaks was observed obviously at the Gr–SP–Tyr/GCE with an oxidation peak at 0.14 V and a reduction peak at 0.01 V. When Tyr/GCE was used, a couple of redox peaks was also observed at the similar redox potentials. In the case of Gr–SP/GCE and the unmodified GCE, no obvious redox peaks were observed. These results may indicate that the redox process on the electrode surface is due to the electrochemical reaction of the *o*-diphenol and quinone species, which liberated from the enzymatic reaction of bisphenol A. For catechol and phenol, they also exhibited the same electrochemical behaviors at the Gr–SP–Tyr/GCE. The mechanism of the enzymatic reaction of phenolic compounds on the Gr–SP–Tyr/GCE surface was shown in Fig. S7 (Wang et al., 2008; Wu et al., 2012).

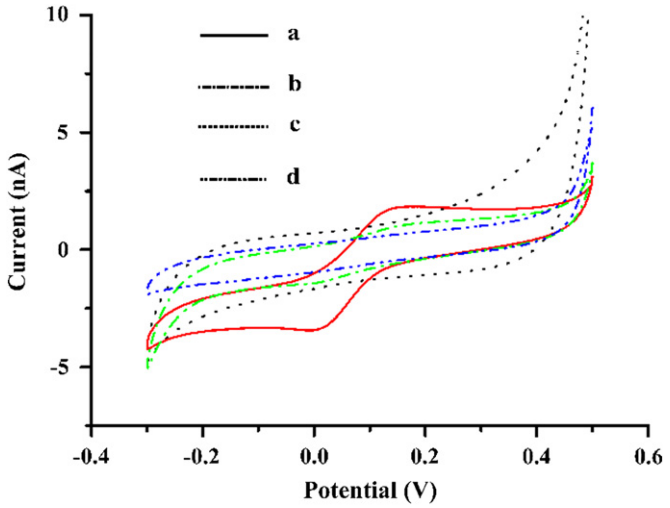

Fig. 2. Cyclic voltammograms of 0.1 mM BPA at the Gr–SP–Tyr/GCE (a), Tyr/GCE (b), Gr–SP/GCE (c) and the unmodified GCE (d).

Furthermore, when using the electrode of Tyr/GCE, peak currents of phenolic compounds were clearly lower than that obtained with the Gr–SP–Tyr/GCE. The increase of the peak current is undoubtedly due to the inherited characteristics of Gr–SP nanosheets such as nanostructure, superior conductivity and biocompatibility. These characteristics led to a high loading of tyrosinase and retaining its biocatalytic activity which can facilitate the enzymatic reaction towards phenolic compounds and thus enhance the current response.

3.3. Optimization of experimental conditions

Bisphenol A was used as a model to investigate the optimum conditions for the amperometric determination of phenolic compounds. Figs. S8 and S9 presented the optimal conditions for the amperometric determination of 0.20 μM BPA. A volume of 5.0 μL Gr–SP–Tyr solution at the tyrosinase/Gr–SP ratio of 8 was used to fabricate biosensor. Amperometric measurements were operated at the applied potential of −0.10 V in PBS at pH 6.0 under 35 °C. As bisphenol A, catechol and phenol possess the similar electroactive groups and biocatalytic reaction, thus, the optimized parameters based on BPA would be suitable for other phenolic compounds.

3.4. Analytical characteristics

The amperometric response of catechol, phenol and BPA was investigated to evaluate the analytical performance of the Gr–SP–Tyr biosensor. Fig. 3(A) shows typical current–time response curves recorded at the Gr–SP–Tyr/GCE biosensor with the successive additions of catechol (a), phenol (b) and BPA (c). Seen from Fig. 3(B), the fabricated Gr–SP–Tyr biosensor exhibits an enzymatic response rapidly and sensitively even though 1.0 nM catechol, 1.5 nM phenol and 2.0 nM BPA were injected into the

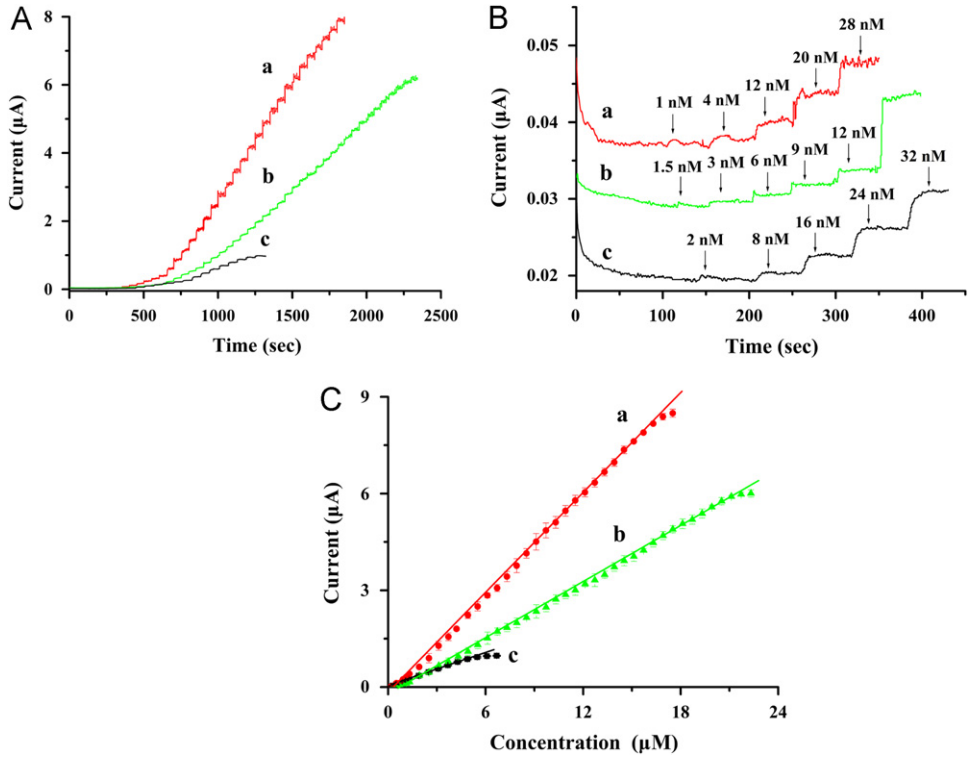

Fig. 3. (A) Typical amperometric response curves for the successive additions of catechol (a), phenol (b) and BPA (c) in phosphate buffer solution (pH 6.5) at the Gr–SP–Tyr biosensor with an applied potential of −0.1 V. (B) Enlarge the amperometric response curves of catechol (a), phenol (b) and BPA (c) at low concentration; (C) calibration curves for the determination of catechol (a), phenol (b) and BPA (c) (standard deviation for $n=3$).

solution. A steady-state current was obtained within 10 s after each addition of phenolic compounds, which indicated that phenolic compounds rapidly diffused from the solution to the Gr–SP–Tyr nanointerface and thus were converted to the quinone species followed by electrochemical reduction at an applied potential of −0.1 V. The calibration plots of the Gr–SP–Tyr biosensors to phenolic compounds were displayed in Fig. 3(C). Analytical characteristics of the Gr–SP–Tyr biosensors to phenolic compounds were listed in Table S2. The linear dynamic range and correlation coefficient of catechol (a), phenol (b) and BPA (c) on the Gr–SP–Tyr biosensor were 0.001–16.91 μM (R=0.9994), 0.0015–21.12 μM (R=0.9993) and 0.002 to 5.48 μM (R=0.9988), respectively. The sensitivity of the developed biosensor was 7634, 4082 and 2511 mA M^{-1} cm^{-2} for catechol, phenol and bisphenol A, respectively. The low detection limit for catechol, phenol and bisphenol A was estimated to be 0.23, 0.35 and 0.72 nmol L^{-1} (S/N=3), respectively.

The performance of the Gr–SP–Tyr biosensor was compared with those of reported biosensors (Table S3). It can be found that the Gr–SP–Tyr biosensor exhibited a wider linear range in the detection of phenolic compounds. In addition, the sensitivity of Gr–SP–Tyr biosensor to BPA, catechol and phenol is significantly greater than those of biosensors. Additionally, the Gr–SP–Tyr biosensor gave a lower detection limit for catechol, phenol and BPA. The results suggested that Gr–SP nanointerface has great potential in the fabrication of biosensors.

The reproducibility was ascertained by monitoring the amperometric current response of 0.20 μM BPA for five times successive injections. The obtained relative standard deviation (RSD) is found to be 3.85%. The fabrication reproducibility was also investigated through the determination of 0.20 μM BPA solutions with six different freshly prepared Gr–SP–Tyr/GCEs. A RSD of 4.60% was obtained. The results indicated that Gr–SP nanosheets provided a reproducible biocompatible interface for the immobilization of tyrosinase to fabricate a biosensor for BPA. The stability of the biosensor was studied by storing at 4 °C in a refrigerator. For a storage period of twenty days, the amperometric response current of 0.2 μM BPA at the biosensor retained 95.4% of its initial response, and decreased to 93.6% over storing for one month. The acceptable stability is due to the Gr–SP nanosheets possess a biocompatible microenvironment to maintain the enzymatic activity of tyrosinase effectively.

The amperometric responses of the biosensor to some potential interferences were investigated to study its specific properties. As shown in Table S4, for the measurement of 0.2 μM BPA, 2.0 μM of vitamin C, uric acid, *m*-dihydroxybenzene and *p*-nitrophnol have no interference. However, in the case of dopamine, catechol and phenol, the biosensor shows good amperometric response, indicating positive interference on the BPA determination. This means that the biosensor shows potential applications in the determination of these phenolic compounds.

To evaluate its practical utility, the proposed biosensor was employed to determine trace amount of BPA in plastic drinking bottle samples with standard addition method. The calculated BPA concentration for drinking bottle sample solution is 6.89 μM (RSD=3.80%, n=3). To further demonstrate the accuracy of the biosensor, high performance liquid chromatography was also employed to analysis BPA in the samples. The obtained BPA concentration is 6.65 μM (RSD=2.86%, n=3). The relative deviation between the result determined with the biosensor and HPLC is 1.77%. As indicated by the results, the developed biosensor is proved to be a promising and reliable tool for the determination of BPA leaching from plastic drinking bottle, and possess potential applications in the analysis of the relevant real samples.

4. Conclusion

Gr–SP nanosheets were synthesized and fully characterized. A novel tyrosinase biosensor based on Gr–SP nanosheets was also successfully developed for the amperometric determination of phenolic compounds. Gr–SP nanosheets provided a suitable interface for high loading of tyrosinase and maintaining its biocatalytic activity due to large area surface and inherit biocompatibility. The resulted biosensor exhibited good analytical performance to phenolic compounds in terms of wide linear range, low detection limit, high sensitivity and stability. The preliminarily practical application in the determination of BPA leaching from plastic drinking bottle was also succeeded with satisfying results. Gr–SP nanosheets may be extended to immobilize other enzymes and bioactive macromolecules to fabricate biosensors. Some studies are under way to facilitate the future application of Gr–SP nanosheets in the fabrication of biosensors.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2013.01.011.

References

Ang, P.K., Chen, W., Wee, A.T.S., Loh, K.P., 2008. Journal of the American Chemical Society 130, 14392–14393.
Chung, K., Lee, C.H., Yi, G.C., 2010. Science 330, 655–657.
Huang, Y., Liang, J.J., Chen, Y.S., 2012. Small 8, 1805–1834.
Laaksonen, P., Kainlauri, M., Laaksonen, T., Shchepetov, A., Jiang, H., Ahopelto, J., Linder, M.B., 2010. Angewandte Chemie International Edition 49, 4946–4949.
Liu, M., Zhao, H.M., Chen, S., Yu, H.T., Zhang, Y.B., Quan, X., 2011. Biosensors and Bioelectronics 26, 4111–4116.
Liu, Y.X., Dong, X.C., Chen, P., 2012. Chemical Society Reviews 41, 2283–2307.
Luo, Z.M., Yuwen, L.H., Han, Y.J., Tian, J., Zhu, X.R., Weng, L.X., Wang, L.H., 2012. Biosensors and Bioelectronics 36, 179–185.
Novoselov, K.S., Geim, A.K., Morozov, S.V., Jiang, D., Zhang, Y., Dubonos, S.V., Grigorieva, I.V., Firsov, A.A., 2004. Science 306, 666–669.
Novoselov, K.S., Jiang, Z., Zhang, Y., Morozov, S.V., Stormer, H.L., Zeitler, U., Maan, J.C., Boebinger, G.S., Kim, P., Geim, A.K., 2007. Science 315, 1379 1379.
Pumera, M., 2011. Materials Today 14, 308–315.
Qi, X., Pu, K.Y., Li, H., Zhou, X., Wu, S., Fan, Q.L., Liu, B., Boey, F., Huang, W., Zhang, H., 2010. Angewandte Chemie International Edition 49, 9426–9429.
Shan, C., Yang, H., Han, D., Zhang, Q., Ivaska, A., Niu, L., 2009. Langmuir 25, 12030–12033.
Sheng, Z.H., Zheng, X.Q., Xu, J.Y., Bao, W.J., Wang, F.B., Xia, X.H., 2012. Biosensors and Bioelectronics 34, 125–131.
Standley, B., Mendez, A., Schmidgall, E., Bockrath, M., 2012. Nano Letters 12, 1165–1169.
Wang, H.B., Chen, T.T., Wu, S., Chu, X., Yu, R.Q., 2012. Biosensors and Bioelectronics 34, 88–93.
Wang, S.F., Tan, Y.M., Zhao, D.M., Liu, G.D., 2008. Biosensors and Bioelectronics 23, 1781–1787.
Wu, L.D., Deng, D.H., Jin, J., Lu, X.B., Chen, J.P., 2012. Biosensors and Bioelectronics 35, 193–199.
Xu, Y., Bai, H., Lu, G., Li, C., Shi, G., 2008. Journal of the American Chemical Society 130, 5856–5857.
Yang, H., Shan, C., Li, F., Han, D., Zhang, Q., Niu, L., 2009. Chemical Communications, 3880–3882.
Zhu, L.M., Luo, L.Q., Wang, Z.X., 2012. Biosensors and Bioelectronics 35, 507–511.