Fabrication of Electroactive Layer-by-layer Films with Myoglobin and Zirconium Phosphate Nanosheets

Xiushuang Yang, Xu Chen, Jiahui Zhang, and Wensheng Yang*
State Key Laboratory of Chemical Resource Engineering, Beijing University of Chemical Technology, Beijing 100029, P. R. China

(Received October 29, 2007; CL-071190; E-mail: yangws@mail.buct.edu.cn)

Electroactive multilayer films composed of exfoliated nanosheets of α-zirconium phosphate and myoglobin (Mb) have been fabricated on the surface of glassy carbon electrodes through electrostatic layer-by-layer self-assembly technique. Direct electron-transfer process of Mb immobilized in the film was investigated.

Recently, a new class of nanomaterials, nanosheets, have been fabricated via delamination of layered compounds.[1,2] These nanosheets exhibit colloidal and polyelectrolytic natures as well as new or enhanced physicochemical properties. Apart from their importance as a new class of nanoscale materials, nanosheets are favorable as building blocks to fabricate functional layer-by-layer (LBL) films.[3] Multilayer films such as polycation/nanosheet,[4] inorganic ion/nanosheet,[5] and nanosheet/nanosheet[6] films have been fabricated to develope the desired functions for various applications. However, direct assembly of redox proteins and nanosheets has not been achieved yet.

During the past few years, the direct electron-transfer reactions between heme proteins and electrodes have been extensively studied which not only can provide the information to understand the electron-transfer processes in the biological systems but also can establish a foundation to prepare biosensors and bioanalytical devices. Myoglobin (Mb) is an ideal model for the study of electron-transfer reaction of heme protein because of its commercial availability, a known and documented structure, and enzyme-like oxidative and reductive catalytic activity. The isoelectric point (pI) of Mb is 6.8,[7] and at appropriate pH Mb molecules can have negative or positive charges and be used as units for electrostatic LBL assembly.

In the present work, electronegative zirconium phosphate nanosheets (ZrPNSs) were selected to immobilize Mb because of their good biocompatibilities, hydrophilic surfaces, thermal stabilities, and chemical inertness.[8] The alternate adsorption of negatively charged ZrPNS and positively charged Mb on electrode surface was used to assemble $\{Mb/ZrPNS\}_n$ LBL films through the electrostatic attraction between them. The direct electrochemistry of Mb at $\{Mb/ZrPNS\}_n$-modified glassy carbon electrode (GCE) was studied.

The precursor layered α-zirconium phosphate (α-ZrP) and ZrPNS were prepared as described in Supporting Information.[15] Field emission scanning electron micrograph (FESEM) image, atomic force micrograph (AFM) image, and X-ray powder diffraction (XRD) patterns show that the precursor layered α-ZrP has been synthesized and delaminated to ZrPNS (See Figures S1–S3).[15] For electrochemical studies, the polished GCE was first immersed into a poly(sodium 4-styrenesulfonate) (PSS) solution (3 mg mL^{-1}) for 20 min to adsorb negatively charged PSS as a precursor layer. The electrode was then alternately immersed for 20 min in Mb solution (3 mg mL^{-1} at pH 5.0) and as-prepared ZrPNS colloid with intermediate water washing and nitrogen stream drying, forming an Mb/ZrPNS bilayer. This cycle was repeated to obtain the desired number (n) of bilayers for $\{Mb/ZrPNS\}_n$ LBL films.

Electrochemical impedance spectra (EIS) were employed to monitor the growth of $\{Mb/ZrPNS\}_n$ films with the redox probe of $Fe(CN)_6^{3-/4-}$ at its formal potential. Figure 1 shows the impedance spectra in the form of Nyquist diagrams at the $\{Mb/ZrPNS\}_n$ film electrodes with different number (n) of bilayers. The typical semicircles were observed for the $\{Mb/ZrPNS\}_n$ films in EIS responses, showing that the LBL buildup of the films had a marked influence on their EIS responses. The diameter of the semicircle usually equals the electron-transfer resistance (R_{ct}), which controls the electron-transfer kinetics of the redox probe at the electrode interface.[9,15] The R_{ct} values showed a nearly linear increase with n for $\{Mb/ZrPNS\}_n$ films (shown in the right corner of Figure 1), suggesting a uniform growth of the films in the assembling process.

Figure 2 shows the FESEM image of $\{Mb/ZrPNS\}_2$ film. ZrPNS tends to orient in the same direction and parallel to the substrate. Edges of ZrPNS overlap with each other and are faintly recognized. So a smooth film without cracks could be formed as reported in the literatures.[10,11] The film thickness of each bilayer in this work was estimated to be about 10 nm from the cross-section FESEM image of $\{Mb/ZrPNS\}_{10}$ in Figure S5.[15]

The electrochemical behavior of $\{Mb/ZrPNS\}_n$-film-modified GCE was studied by cyclic voltammetry (CV). $\{Mb/ZrPNS\}_2$/GCE shows well-defined, nearly reversible cyclic voltammetric peaks for Mb heme Fe^{III}/Fe^{II} redox couples (Figure 3a). There are no redox peaks for ZrPNS/GCE or bare GCE within the potential window (−0.8 to 0.1 V vs. Ag/AgCl) in the same solution (Figures 3b and 3c). The inefficient electron

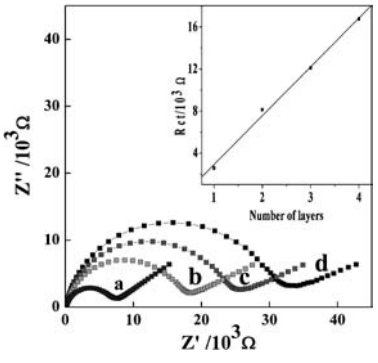

Figure 1. EIS responses of $\{Mb/ZrPNS\}_n$ films with $n = 1$–4 (a–d) in 5 mM $Fe(CN)_6^{3-/4-}$ solution. Influence of n on R_{ct} for $\{Mb/ZrPNS\}_n$ films is shown in the right corner.

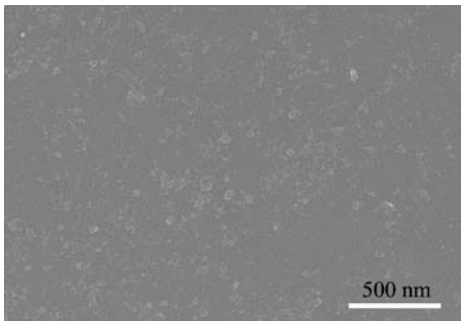

Figure 2. FESEM image of $\{Mb/ZrPNS\}_2$ film.

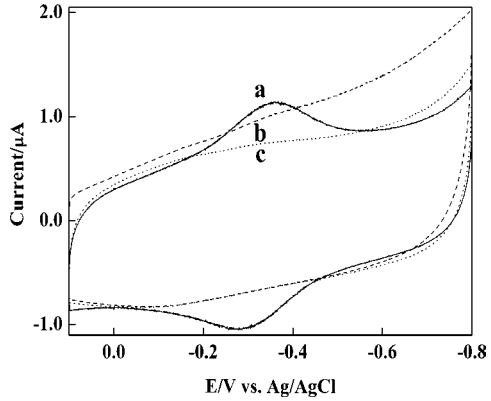

Figure 3. Cyclic voltammograms obtained with $\{Mb/ZrPNS\}_2$/GCE (a), ZrPNS/GCE (b), and bare GCE (c) in 0.1 M phosphate buffer solution (pH 7.0). Scan rate: 0.1 V s^{-1}.

transfer of hemoglobin immobilized in α-ZrP nanoparticle film has been observed by Chen et al. probably owing to the lower enzyme-loading ability of α-ZrP nanoparticles.[12] Although the exact reason that $\{Mb/ZrPNS\}_n$ films can effectively enhance the electron exchange of Mb is not clear yet, it probably arises from the unique specialty of ZrPNS. On one hand, ZrPNS provides large surface area upon exfoliation from α-ZrP,[8] which may provide a high enzyme loading. On the other hand, the electron transfer is probably facilitated by a charge-hopping mechanism as suggested for polyion/protein films.[13] The polyelectrolytic nature of ZrPNS possibly enhances the electron transfer between Mb and the underlying electrodes. The reasons described above make the Mb in $\{Mb/ZrPNS\}_n$ films transfer electron effectively with GCE. In addition, the stability of the electroactive film has been studied. The peak current of $\{Mb/ZrPNS\}_2$/GCE has not decreased for forty cycles.

When $n = 2$, the peak currents reached the largest value and essentially decreased with n. The $\{Mb/ZrPNS\}_2$ film was thus used for the following voltammetric studies. Both reduction and oxidation peak currents of $\{Mb/ZrPNS\}_2$/GCE increased linearly with scan rates in the range from 0.05 to 1.0 V s^{-1}, indicating a surface-confined electrode process. The average electron-transfer rate constant (k_s) is 7.79 s^{-1}, estimated by the method of Laviron.[14] In this kind of system, the surface concentration of electroactive proteins (Γ^*) can be estimated by integration of CV reduction peak and applying the equation of $Q = nFA\Gamma^*$, The Γ^* value for $\{Mb/ZrPNS\}_2$ films is estimated to be 8.6×10^{-11} mol cm^{-2}. This value is close to that of Mb assembled with PPI–Au nanoclusters.[13]

The electrocatalytic behavior of $\{Mb/ZrPNS\}_2$ LBL film has also been studied (see Figure S6).[15] The influence of the number of bilayers (n) for $\{Mb/ZrPNS\}_n$ films on the i_{pc} values in the same conditions is shown in the inset of Figure S6.[15] The electroactive-film-modified GCE possesses good electrocatalytic reduction ability to H_2O_2.

In conclusion, a novel $\{Mb/ZrPNS\}_n$ film has been fabricated by LBL assembly method for the first time. Direct electron transfer of Mb is realized in $\{Mb/ZrPNS\}_n$ film. The $\{Mb/ZrPNS\}_n$ films may have a potential application in fabricating the third-generation biosensors based on mediator-free electrochemistry of the proteins. The combination of the novel nanomaterial—nanosheets and proteins through LBL assembly method can provide a promising platform for fabricating other electroactive films and then preparing biosensors and bioreactors.

The authors gratefully acknowledge financial support from the National 863 Project (Grant No. 2006AA03Z343), the National Natural Science Foundation of China, the 111 Project (Grant No. B07004), and Program for Changjiang Scholars and Innovative Research Team in University (Grant No. IRT0406).

References and Notes

1 F. Carn, A. Derré, W. Neri, O. Babot, H. Deleuze, R. Backov, *New J. Chem.* **2005**, *29*, 1346.
2 Y. Omomo, T. Sasaki, L. Wang, M. Watanabe, *J. Am. Chem. Soc.* **2003**, *125*, 3568.
3 T. Sasaki, *J. Ceram. Soc. Jpn.* **2007**, *115*, 9.
4 L. Wang, Y. Omomo, N. Sakai, K. Fukuda, I. Nakai, Y. Ebina, K. Takada, M. Watanabe, T. Sasaki, *Chem. Mater.* **2003**, *15*, 2873.
5 L. Wang, N. Sakai, Y. Ebina, K. Takada, T. Sasaki, *Chem. Mater.* **2005**, *17*, 1352.
6 L. Li, R. Ma, Y. Ebina, K. Fukuda, K. Takada, T. Sasaki, *J. Am. Chem. Soc.* **2007**, *129*, 8000.
7 W. Guo, N. Hu, *Biophys. Chem.* **2007**, *129*, 163.
8 C. V. Kumar, A. Chaudhari, *J. Am. Chem. Soc.* **2000**, *122*, 830.
9 E. Katz, I. Willner, *Electroanalysis* **2003**, *15*, 913.
10 L. Wang, C. Li, M. Liu, D. G. Evans, X. Duan, *Chem. Commun.* **2007**, 123.
11 H. Xiao, X. Chen, L. Ji, X. Zhang, W. Yang, *Chem. Lett.* **2007**, *36*, 772.
12 J.-J. Feng, J.-J. Xu, H.-Y. Chen, *J. Electroanal. Chem.* **2005**, *585*, 44.
13 H. Zhang, N. Hu, *Biosens. Bioelectron.* **2007**, *23*, 393.
14 E. Laviron, *J. Electroanal. Chem.* **1979**, *101*, 19.
15 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/.

Published on the web (Advance View) February 2, 2008; doi:10.1246/cl.2008.240