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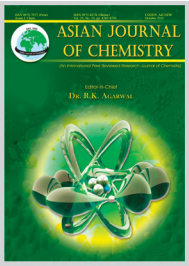

Electrochemical Study of the Supported Bilayer Lipid Membrane Doped with Ionic Liquids and the Direct Electron Transfer of Embed Laccase

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The electrochemical behaviour of ionic liquids doped supported bilayer lipid membrane on glassy carbon electrode was studied by cyclic voltammetry. The assembled supported bilayer lipid membrane/ionic liquids films exhibited good electrochemical performance. The direct electron transfer of immobilized laccase was observed through the electrocatalytical ability toward the reduction of oxygen with 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulphonate) as an electron mediator. In addition, the supported phospholipid layers incorporated with ionic liquids could be come true in the direct electron transfer. Potential electroanalytical applications and the development of biosensors will be explored in the future.

Key Words: Cyclic voltammetry, Ionic liquid, Supported bilayer lipid membrane, Laccase, Direct electron transfer.

INTRODUCTION

In the early 1990s, researchers began to interest in exploring the electrochemistry propertites of small electroactive molecules incorporated with multi-bilayer-film materials which exhibited excellent behaviour of charge transportation. Subsequently, Rusling *et al.*[1] studied the electrochemical behaviour of cast lipid film with incorporated protein. Yamada *et al.*[2] also investigated the transmembrane electron transfer properties by using bilayer lipid membrane modified with 7,7,8,8-tetracyanoquinodimethane through AC impedance spectroscopy characterization.

The supported bilayer lipid membrane (s-BLM) provides a highly biomimic surface microenvironment, which can be reacted with various biocomponents such as proteins, pigments under non-denaturing conditions with a well-defined orientation[3,4]. Consequently, the lipid film was an ideal candidate for developing a new class of biosensor, which was widely used to study the structure and properties of native biological membranes and to investigate the biological processes.

However, there is still a big challenge for using supported membranes: the lipid bilayer film acts as a very thin electric insulator, which is a disadvantage for the conduction of ions and electrons. Thus, considerable interests arised in achieving direct electron transfer between enzyme and the electrode surface for the applications of biosensors, chemical synthesis and fundamental studies.

More recently, ionic liquids have received much attention in electrochemical field. Ionic liquid not only can be used as the supporting solvent electrolyte, but also can be used in the dissolution of a large number of single-walled carbon nanotubes for the preparation of carbon nanotubes ionic liquid film modified electrodes[5]. Furthermore, on account of the high conductivity[6,7], ionic liquid could accelerate the electron transfer.

To our best of knowledge, only few studies have dealt with the impact of direct electrochemistry of supported bilayer lipid membrane doped with ionic liquids. In this paper, the mixture of ionic liquids and lecithin were dropwised onto the glassy carbon electrode to attain a film which is favourable for direct electron transfer with some redox proteins on the electrode. As an industrially attractive enzyme, laccase is able to catalyze electron transfer reactions without additional ingredients or the formation of reactive intermediates[8] which makes laccase can be used as a powerful tool for the electrochemical applications *e.g.*, as an oxidant of biosensor construction[9-12], phenolic substrates[13] and electrocatalytic dioxygen reduction applied in biofuel cells[14,15]. Consequently, laccase was selected as the model enzyme and further studies confirmed its good activity.

EXPERIMENTAL

1-Ethyl-3-methylimidazolium hexafluorophosphate was purchased from Shanghai Chengjie Chemical Co., Ltd. (Shanghai, China). Lecithin was obtained from Aladdin Reagent Database Inc. (Shanghai, China). The acetate buffer contained 50 mM CH_3COOH, 50 mM CH_3COONa and 0.1 M

KCl. Laccase (trametes versicolour) was from Biochemika. Ultra-pure water was prepared from Milli-Q set. All chemicals were of analytical grade and used without further purification.

Preparation of the samples: A glassy carbon electrode was polished with 0.5 μm alumina slurry, respectively and then sonicated in ultra-pure water for 10 s and dipped into HNO_3 : H_2O (1:1) for 1 min. Subsequently, the electrode was immersed in a 0.1 M NaOH solution and the potential was held at 1500 mV for 3 min to polarize the electrode. After the glassy carbon electrode was polarized, it was dried under a purified nitrogen stream at room temperature[16].

Liposomes were prepared by dissolving different proportions of lecithin and ionic liquids in ethanol followed by drying under nitrogen to allow the solvent vapourizing. Then ultra-pure water was added into the system and the mixture was sonicated at 60 ºC for 15 min to obtain a final solution with concentration of 2.5 mg mL^{-1} [17-19], which was called s-BLM/ ionic liquids/formation solution. Aliquot of the mixture (8 μL) was dropped onto the surface of the glassy carbon electrode by using a microsyringe. The as-prepared film was self-assembled under high humidity for 6 h at 4 ºC, followed by water rinsing for several times. After dried for 1 h, the obtained Lecithin-ionic liquids-modified electrode was defined as ionic liquids/supported-bilayer lipid membrane/glassy carbon electrode, which were further modified with 8 μL laccase (0.1 mg mL^{-1}) for 17 h at 4 ºC and then used for electrochemical characterization.

Electrochemical measurements: Cyclic voltammograms (CVs) was performed by using CHI 760 electrochemical analyzer (CH Instruments, Shanghai, China). All experiments were conducted with a three-electrode system consisting of an Ag/AgCl (KCl saturated) electrode as the reference electrode, a platinum coil as the auxiliary electrode and a glassy carbon electrode as the working electrode. Electrochemical measurement was performed in the presence of a 1 mM $K_3[Fe(CN)_6]$/ $K_4[Fe(CN)_6]$ (1 : 1) mixture containing 0.1 M KCl solution.

RESULTS AND DISCUSSION

The study of interaction between ionic liquids and supported bilayer lipid membrane was investigated with $Fe(CN)_6^{3-/4-}$ as the marker ions. The amperometric response of the marker ions on supported bilayer lipid membrane at different concentrations of ionic liquids was shown in Fig. 1a (a-d), we concluded that the $Fe(CN)_6^{3-}$ ions could be prevented from reaching the surface of glassy carbon electrode to a certain degree, which implied that the supported bilayer lipid membrane had been successfully formed on the surface of electrode. With the concentration of ionic liquids increased, the s-BLM/ionic liquids/glassy carbon electrode has an increased amperometric response and a decrease in the peak-to-peak separation between the cathodic and anodic waves of $Fe(CN)_6^{3-}$. When the concentration of incorporation ionic liquids in lipid reached 1 mg mL^{-1} in Fig. 1a (a), the redox response reached a plateau without further change and its shape was almost the same with that obtained on bare electrode (inset of Fig. 1a). It is clear that ionic liquids can stimulate the ion channels of the membranes to an open state. Thus, the incorporation of ionic liquids (1-ethyl-3-methylimidazolium hexafluorophosphate) could not only stabilize but also effectively accelerate the electron transfer process.

Fig. 1b showed that the amperometric response of laccase gradually enhanced with increasing the concentration of incorporated ionic liquids. When the mass concentration ratio of ionic liquids in s-BLM/ionic liquids/formation solution was 8/20, the amperometric response was the best. A pair of redox peaks were observed at 0.28 V and 0.39 V respectively, the formal potential is around 0.33 V. The redox peaks are attributed to the redox reactions of immobilized laccase on s-BLM /ionic liquids modified electrode. It can be seen that the ionic liquids played an important role in improving the laccase immobilization and facilitated the direct electron transfer between laccase and glassy carbon electrode.

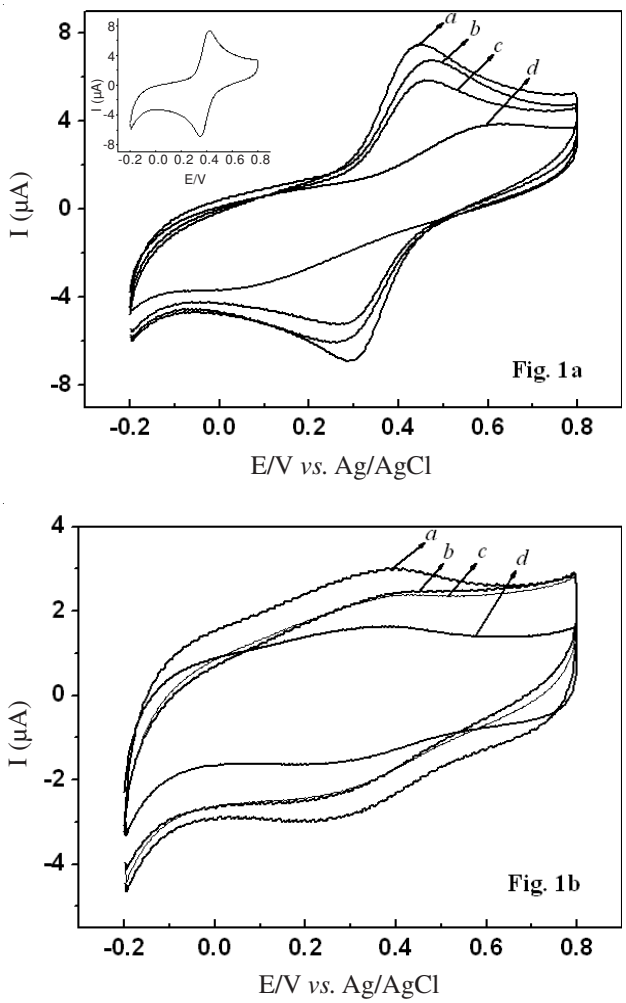

Fig. 1. Cyclic voltammetric response obtained with the modified glassy carbon electrode (GCE). Fig. 1a: s-BLM/ionic liquids/glassy carbon electrode in 1 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) solution containing 0.1 M KCl. The concentration of the incorporation ionic liquids was respectively (a) 1.0 mg mL^{-1}; (b) 0.5 mg mL^{-1}; (c) 0.25 mg mL^{-1}; (d) 0 mg mL^{-1}. Inset: cyclic voltammetry response on the bare glassy carbon electrode. Scan rate: 100 mV s^{-1}. Fig. 1b: s-BLM/ionic liquids/Laccase/glassy carbon electrode in 0.05 M acetate buffer (pH 5.0). The mass concentration ratios of ionic liquids in liposome were as follows: (a) 8/20; (b) 4/20; (c) 2/20; (d) 0/20. Scan rate: 100 mV s^{-1}.

The influence of the scan rate on the cyclic voltammetry performance of the immobilized laccase has been investigated,

(Fig. 2a). The redox reactions of the s-BLM/ionic liquids/laccase gave roughly symmetric anodic and cathodic peaks at relatively slow scan rates, suggesting facile charge transfer kinetics going through and somewhat surface-confined redox process. With the scan rate increased, the anodic peak potential of laccase shifted to a more positive value and the cathodic peak potential shifted in a negative direction, the ΔE_p also increased.

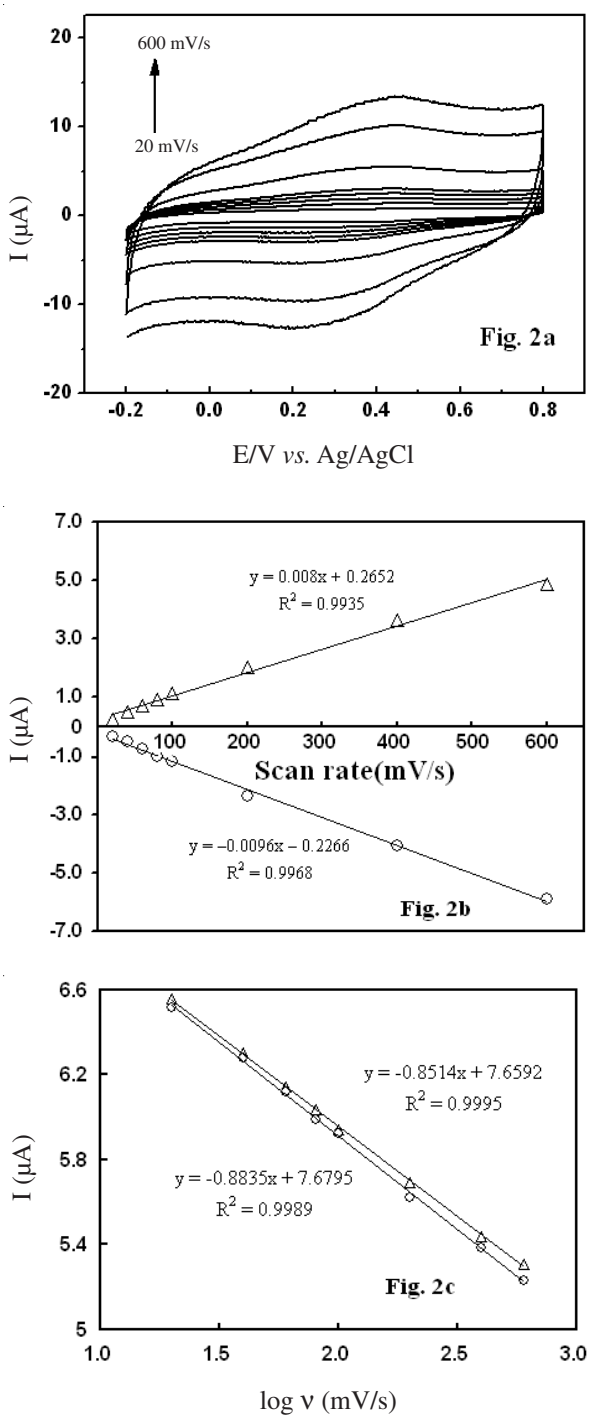

Fig. 2. Cyclic voltammograms of the modified glassy carbon electrode with s-BLM/ionic liquids/laccase film in acetate buffer (pH 5.0) with 0.1 M KCl at different scan rates: 20, 40, 60, 80, 100, 200, 400 and 600 mV s^{-1} (Fig. 2a); The plot of the peak current vs. scan rates (Fig. 2b); And the relationship of the peak current vs. the logarithm of scan rate (Fig. 2c)

At the same time, the plot of the peak current on scan rates displayed in Fig. 2b exhibits a linear dependence, as expected to be a surface confined electrode reaction. Fig. 2c also shows the linear relationship of anodic and cathodic peak currents *vs.* logarithm of scan rate with slopes of 0.8514 and 0.8835, which are closer than the slope of 1 for ideal thin layer electrochemistry[20]. All these characteristics suggested that the redox reaction of the laccase on s-BLM/ionic liquids film modified electrode is an adsorption-controlled electrochemical process[21,22]. The small peak-to-peak separation indicated a fast electron transfer rate[23].

The activity center of laccase has four copper ions, according to spectroscopy, it can be divided into three kinds of characteristics[24]: T1, T2 and two T3 copper. As the relatively independent of T1 copper center, the oxidation-reduction potential become a important parameter in the biological fuel cell applications, which decide to the battery voltage[25]. In order to help in the regeneration of the reduced Cu^+ form of the enzyme, the common mediator 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulphonate) has been added to the solution to make the electron transfer more efficiently at the T1 site. Principle as shown in Fig. 3.

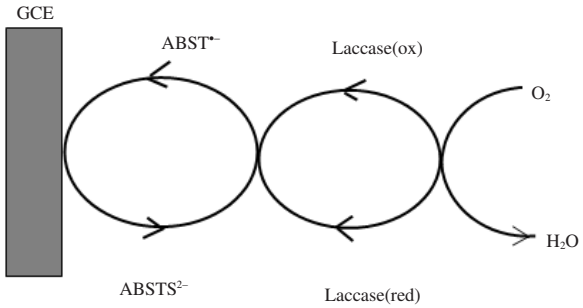

Fig. 3. Scheme of electrocatalytic reduction of oxygen by s-BLM/ionic liquids/laccase electrode

The catalytic reaction starts immediately when enzyme, mediator and substrate are mixed, namely at the moment of the electrode immersed in the electrolyte solution. The voltammograms of s-BLM/ionic liquids/laccase/glassy carbon electrode in acetate buffers (pH 5.0) containing 0.2 mM 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulphonate) (absence and presence of oxygen) was shown in Fig. 4. The redox peaks of 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulphonate) appeared at 0.56 and 0.67 V respectively, these data are well agreement with the literature. Compared to the deoxygenated results under nitrogen, a distinctive decrease of the cathodic current and a slight distinctive increase in the anodic current showed up in the oxygen saturated solution[26-28]. The redox mediator 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulphonate) is oxidized by laccase and the regeneration of the enzyme is achieved by the reduction of oxygen molecular to water which indicated that the s-BLM/ionic liquids film exhibits excellent electrocatalytic activity.

Conclusion

Iionic liquids were successfully incorporated into supported phospholipid layers on glassy carbon electrode. The obtained s-BLM/ionic liquids/glassy carbon electrode

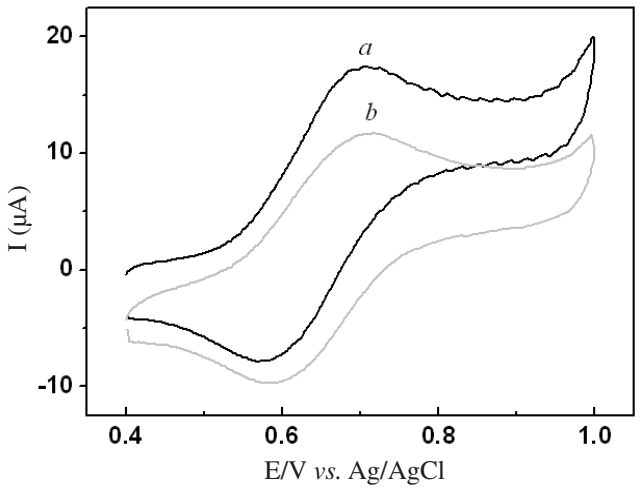

Fig. 4. Cyclic voltammograms of the s-BLM/ionic liquids/laccase/glassy carbon electrode in acetate buffer (pH 5.0) containing 0.2 mM, 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulphonate). Solution deoxygenated with N_2 (a) and saturated with O_2 (b). Scan rate: 100 mV s^{-1}

exhibited good electrochemical response and stability, also showed well electrocatalytical activity toward laccase. The stable s-BLM/ionic liquids films on glassy carbon electrode gave an excellent model for constructing the direct electron transfer biosensor.

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REFERENCES

1. L.F. Rusling and A.E.F. Nassar, *J. Am. Chem. Soc.*, **115**, 11891 (1993).
2. H. Yamada, H. Shiku, T. Matsue and I. Uchida, *J. Phys. Chem.*, **97**, 9547 (1993).
3. L. Faxälv, J. Hume, B. Kasemo and S. Svedhem, *J. Coll. Interf. Sci.*, **364**, 582 (2011).
4. M. Zviman and H.T. Tien, *Biosens. Bioelectron.*, **6**, 37 (1991).
5. P. Kubisa, *Prog. Polym. Sci.*, **34**, 1333 (2009).
6. A. Jarosik, S.R. Krajewski, A. Lewandowski and P. Radzimski, *J. Mol. Liq.*, **123**, 43 (2006).
7. S.K. Chaurasia, R.K. Singh and S. Chandra, *Solid State Ionics*, **183**, 34 (2011).
8. E.I. Solomon, U.M. Sundaram and T.E. Machonkin, *Chem. Rev.*, **96**, 2563 (1996).
9. L. Xiang, Y.Q. Lin, P. Yu, L. Su and L.Q. Mao, *Electrochim. Acta*, **52**, 4145 (2007).
10. R.S. Freire, N. Durán and L.T. Kubota, *Talanta*, **54**, 686 (2001).
11. J.X. Liu, W.J. Zhou, J.L. Gong, L. Tang, Y. Zhang, H.Y. Yu, B. Wang, X.M. Xu and G.M. Zeng, *Bioresour. Technol.*, **99**, 8748 (2008).
12. A. Jarosz-Wilkolazka, T. Ruzgas and L. Gorton, *Enzyme Microb. Technol.*, **35**, 239 (2004).
13. S. Shleev, P. Persson, G. Shumakovich, Y. Mazhugo, A. Yaropolov, T. Ruzgas and L. Gorton, *Enzyme Microb. Technol.*, **39**, 837 (2006).
14. R.A. Bullen, T.C. Arnot, J.B. Lakeman and F.C. Walsh, *Biosens. Bioelectron.*, **21**, 2035 (2006).
15. F. Davis and S.P.J. Higson, *Biosens. Bioelectron.*, **22**, 1230 (2007).
16. X. Liu, H. Bai, W. Huang, L. Du, X. Yang and E. Wang, *Electrochim. Acta*, **51**, 2512 (2006).
17. Z.Y. Wu, B.Q. Wang, Z.L. Cheng, X.R. Yang, S.J. Dong and E.K. Wang, *Biosens. Bioelectron*., **16**, 47 (2001).
18. Y. Wang, J. Chen, Q. Ren and J. Miao, *Asian J. Chem.*, **24**, 761 (2012).
19. Z.Y. Wu, J.L. Tang, Z.L. Cheng, X.R. Yang and E.K. Wang, *Anal. Chem.*, **72**, 6030 (2000).
20. Y. Li, E.M.W. Tsang, A.Y.C. Chan and H. Zh. Yu, *Electrochem. Commun.*, **8**, 951 (2006).
21. Y. Miao, J. Chen, X. Wu, K. Fang, A. Jia and J. Liu, *J. Nanosci. Nanotech.*, **7**, 2877 (2007).
22. C.S. Shan, H.F. Yang, J.F. Song, D.X. Han, A. Ivaska and L. Niu, *Anal. Chem.*, **81**, 2378 (2009).
23. Z.H. Dai, X.X. Xu and H.X. Ju, *Anal. Biochem.*, **332**, 29 (2004).
24. U. Sundaram, B. Hedman, K. Hodgson, H. Zhang and E. Slolmon, *J. Am. Chem. Soc.*, **119**, 12525 (1997).
25. V. Soukharev, N. Mano and A. Heller, *J. Am. Chem. Soc.*, **126**, 8368 (2004).
26. I. Zawisza, J. Rogalski and M. Opallo, *J. Electroanal. Chem.*, **588**, 244 (2006).
27. Y. Miao, X. Wu, J. Chen, J. Liu and J. Qiu, *Gold Bull.*, **41**, 336 (2008).
28. E.I. Iwuoha, A.R. Williams and L.A. Hall, *Electroanalysis*, **14**, 1177 (2002).