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Ionic Liquid-Assisted Preparation of Laccase-Based Biocathodes with Improved Biocompatibility

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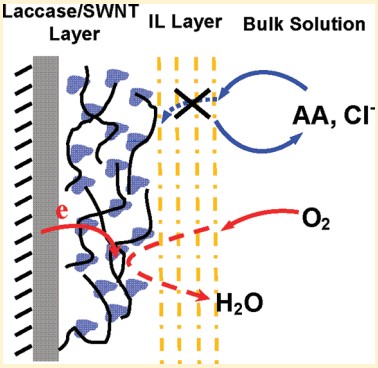
ABSTRACT: Laccase enzyme has been widely used as the catalyst of the biocathodes in enzymatic biofuel cells (BFCs); the poor biocompatibility of this enzyme (e.g., poor catalytic activity in neutral media and low tolerance against chloride ion) and the lack of selectivity for oxygen reduction at the laccase-based biocathode against ascorbic acid, unfortunately, offer a great limitation to future biological applications of laccase-based BFCs. This study demonstrates a facial yet effective solution to these limitations with the assistance of hydrophobic room temperature ionic liquid, 1-butyl-3-methylimidazolium hexafluorophosphate ($Bmim^+PF_6^-$). With the $Bmim^+PF_6^-$ overcoating, the laccase-based biocathodes possess a good bioelectrocatalytic activity toward O_2 reduction in neutral media and a high tolerance against Cl^-. Moreover, the $Bmim^+PF_6^-$ overcoating applied to the laccase-based biocathodes also well suppresses the oxidation of ascorbic acid (AA) at the biocathodes and thereby avoids the AA-induced decrease in the power output of the laccase-based BFCs. The mechanisms underlying the excellent properties of the $Bmim^+PF_6^-$ overcoating are proposed based on the intrinsic features of ionic liquid $Bmim^+PF_6^-$. To demonstrate the applications of the BFCs with the as-prepared biocathodes in biologically relevant systems, an AA/O_2 BFC is assembled with single-walled carbon nanotubes (SWNTs) as electrode materials both for accelerating AA oxidation at the bioanode and for promoting direct electron transfer of laccase at the biocathode. With the presence of 0.50 mM AA in 0.10 M quiescent phosphate buffer (pH 7.2), the assembled BFC has an open circuit voltage of 0.73 V and a maximum power output of 24 $\mu W\ cm^{-2}$ at 0.40 V under ambient air and room temperature. This study essentially offers a new strategy for the development of enzymatic BFCs with a high biocompatibility.



1. INTRODUCTION

Enzymatic biofuel cells (BFCs) represent one kind of molecular bioelectronic devices that convert chemical energy stored in biomass into electrical energy.[1−8] Unlike conventional fuel cells with noble metals as the catalysts, BFCs use enzymes as the catalysts for selective oxidation of biomass and reduction of O_2 to generate electrical power output. The alterative uses of enzyme catalysts actually endow the BFCs with the advantages of a wide variety of possible biofuel sources including alcohols,[9−12] sugars,[13,14] and carboxymethyl cellulose,[15] high selectivity for biofuel oxidation, mild operation conditions (i.e., ambient temperature and neutral pH), and future applications as in vivo power sources with biologically endogenous species as biofuels. Nowadays, BFCs have been a great concern both in the fundamental bioelectrochemical studies and in the practical applications based on biofuel cell technologies.[16−22]

As one of the most important multicopper enzymes (MCOs), laccase (EC1.10.3.2) is an extracellular blue copper enzyme in plants and fungi. This enzyme catalyzes the oxidation of phenols and amines, concomitantly with the four-electron reduction of molecular oxygen into water.[23−28] Spectroscopic and X-ray crystallographic studies have revealed that laccases contain one blue copper or type 1 (T1) site and a T2/T3 (type 2/type 3) trinuclear copper cluster site consisting of a normal copper type 2 (T2) and a bridged copper pair type 3 (T3).[29−33] The cysteine bound to the T1 copper is flanked on either side by histidines that are ligated to each of the T3 coppers, providing a 1.3 nm pathway for electron transfer from the T1 site to the T2/T3 site.[3,34] Electrons acquired by the T1 site from the oxidized cosubstrate are transferred internally by electron tunneling through the cysteine−histidine pathway to the trinuclear site, where oxygen is reduced into water.[35] As one kind of laccases, fungal laccase has higher redox potentials of the T1 copper sites than other blue copper oxidases, which is primarily attributed to nonaxial methionine ligand, a geometry that stabilizes the reduced state.[3,36] Furthermore, it is reported that the direct electron transfer between laccase and electrode could be well promoted by using single-walled carbon nanotubes as electrode materials, which has been used for the development of the biocathodes with a low overpotential for the O_2 reduction.[8,37] While the catalytic ability of laccase enzyme for four-electron reduction of O_2 at unprecedented high potentials has greatly enabled it to be widely used as the cathode catalysts for BFCs, some challenges have to be resolved when the laccase-based BFCs are used in the future biologically related applications. First, fungal laccases normally have optimal activity only in weakly acidic media and lose almost all of their catalytic activity in neutral media. This feature, unfortunately,

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provides a great obstacle to operation of laccase-based BFCs in biological systems with neutral pH values.[38,39] Second, as one kind of anion that is widely distributed in the biological systems, chloride ion can bind to the T2/T3 trinuclear copper cluster of laccase, resulting in the deactivation of this enzyme.[40] Third, since laccase-catalyzed O_2 electroreduction normally occurs at a high potential, some kinds of electroactive species (typically, ascorbic acid) endogenously existing in the biological systems can be readily oxidized at the cathodes, leading to the decrease in the power output of the as-prepared biocathodes.

In this study, we demonstrate an effective solution to these limitations inherent in the laccase-based biocathodes through surface overcoating of hydrophobic ionic liquid $Bmim^+PF_6^-$. The surface overcoating of ionic liquid layer eventually enables the as-prepared laccase-based biocathodes to work efficiently for O_2 reduction in biocompatible solutions with neutral pH and with the presence of Cl^- and ascorbic acid. This study essentially paves a new way to development of new kinds of enzymatic BFCs for future biological applications including in vivo power sources, biotransformation, biosensors, and so on.

2. EXPERIMENTAL SECTION

Chemicals and Reagents. 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) was purchased from Sigma-Aldrich Corp. Single-walled carbon nanotubes (SWNTs, purity, 95%; diameter <2 nm; length 0.5−50 μm) were purchased from Shenzhen Nanotech Port Co., Ltd. (Shenzhen, China). The nanotubes were purified by refluxing the as-received samples in 2.6 M HNO_3 for 10 h, followed by centrifugation, resuspension, filtration, and being dried to evaporate the solvent. Laccase (E.C. 1.10.3.2, initial activity: 21.7 U mg^{-1}) from *Trametes versicolor* was purchased from Sigma-Aldrich and purified with a method described in our earlier work.[41] 1-Butyl-3-methylimidazolium hexafluorophosphate ($Bmim^+PF_6^-$) was synthesized by a metathesis reaction between excess HPF_6 and $Bmim^+Cl^-$, and the as-synthesized $Bmim^+PF_6^-$ was washed by Milli-Q water for several times to remove excess reactants before use.[42] Ascorbic acid and other chemicals were purchased from Beijing Chemical Corp. (Beijing, China). Aqueous solutions were all prepared with Milli-Q water (Millipore).

Preparation of the Laccase-Based Biocathodes. Glassy carbon electrodes (GC, 3 mm diameter) purchased from Bioanalytical Systems Inc. (BAS, West Lafayette, IN) were used as the substrate for preparing the bioanode and biocathode. GC electrodes were first polished with emery paper (# 2000) as well as 0.3 and 0.05 μm alumina slurry on a polishing cloth, then sonicated in acetone and Milli-Q water (each for 3−5 min), and finally thoroughly rinsed with Milli-Q water. SWNTs were dispersed into ethanol to give a homogeneous suspension (2 mg mL^{-1}). A 4 μL of the SWNT dispersion was dip-coated onto GC electrodes with a syringe and the electrodes (SWNT-modified electrodes) were air-dried. Then, 6 μL of the laccase solution was coated onto the SWNT-modified electrodes, and the electrodes (laccase/SWNT-modified electrodes) were air-dried. To prepare the biocathodes with surface overcoated by $Bmim^+PF_6^-$, the laccase/SWNT-modified electrodes were surrounded with a thermoplastic tube to form a small space; 5 μL of 0.10 M phosphate solution (pH 6.0) and 50 μL of $Bmim^+PF_6^-$ were successively dip-coated and held onto the electrode surface.

Electrochemical Measurements. Electrochemical measurements were carried out with a computer-controlled electrochemical analyzer (CHI 660A, CHI, Austin, TX) in a two-compartment and three-electrode cell. Laccase-modified electrodes were used as working electrode and a platinum spiral wire as counter electrode. All potentials employed here were versus Ag/AgCl reference electrode (0.199 V vs NHE). For assembling an ascorbic acid/O_2 BFC, the SWNT-modified electrode was used as the anode for the oxidation of ascorbic acid and the $Bmim^+PF_6^-$/laccase/SWNT-modified electrode was used as the biocathode for the reduction of oxygen. The performance of the ascorbic acid/O_2 BFC was studied by immersing the anode and the biocathode into 0.10 M phosphate solution (pH 7.0) containing 0.50 mM ascorbic acid under ambient air. All measurements were carried out at ambient temperature.

ABTS-Based Assay for Laccase Activity. To clarify whether laccase activity was inhibited or not when laccase was exposed to $Bmim^+PF_6^-$, the ABTS-based assay for activity of laccase was conducted.[43−46] Typically, 20 μL of the ethanol dispersion of SWNTs (2 mg mL^{-1}) was dip-coated onto indium tin oxide (ITO) plates (4.0 cm × 2.5 cm) with a syringe, and the plates were then dried under ambient temperature to prepare SWNT-modified ITO plates. The laccase/SWNT-modified ITO plates were prepared by dip-coating 20 μL of the laccase solution in phosphate buffer (pH 6.0) onto the SWNT-modified ITO plates and allowing the plates (plate 1, for convenient comparison) to dry under ambient temperature. To clarify the protective effect of water layer applied between the layers of laccase and $Bmim^+PF_6^-$ toward laccase, two plates were prepared based on the laccase/SWNT-modified ITO plates. One was prepared by first applying 15 μL of 0.10 M phosphate solution (pH 6.0) and then 20 μL of $Bmim^+PF_6^-$ onto the laccase/SWNT-modified ITO plates (plate 2, hereafter), and the other was prepared by only applying 20 μL of $Bmim^+PF_6^-$ onto the laccase/SWNT-modified ITO plates (i.e., without prior applying of 15 μL of 0.10 M phosphate solution) (plate 3, hereafter). Prior to the ABTS-based assay for the laccase activity, plates 2 and 3 were stored at ambient temperature for 2 h, and the $Bmim^+PF_6^-$ layer overcoated onto the ITO plates was removed with a syringe. The ABTS-based assay was then performed in 0.10 M phosphate solution (pH 6.0, 4.0 mL) containing ABTS (500 μM) with UV−vis spectrometry. UV−vis spectra of ABTS were recorded after the plates were immersed into the ABTS solution for 5 min and taken out of the solution.

3. RESULTS AND DISCUSSION

Figure 1 compares cyclic voltammograms (CVs) obtained at the laccase/SWNT-modified electrodes in the buffers with different pH values. A comparison of the voltammograms obtained in the buffers saturated with N_2 (black curves) or ambient air atmosphere (red curves) indicates that the O_2 reduction occurs in the buffers with different pH values of 4.0 (A), 5.0 (B), and 6.0 (C). As reported in our early studies,[8,20] under the conditions employed here, the O_2 reduction undergoes a bioelectrocatalytic pathway based on a direct electron-transfer mechanism of laccase enzyme at SWNTs. The peak potentials for the bioelectrocatalytic O_2 reduction at the laccase/SWNT-modified electrodes in the buffers with different pH values of 4.0, 5.0, and 6.0 were ca. +0.78 V (A), +0.70 V (B), and +0.60 V (C), respectively. All potentials were versus to Ag/AgCl reference electrode (0.199 V vs NHE). The pH dependence of the potentials for the O_2 reduction suggests that protons are involved in the bioelectrocatalytic reduction

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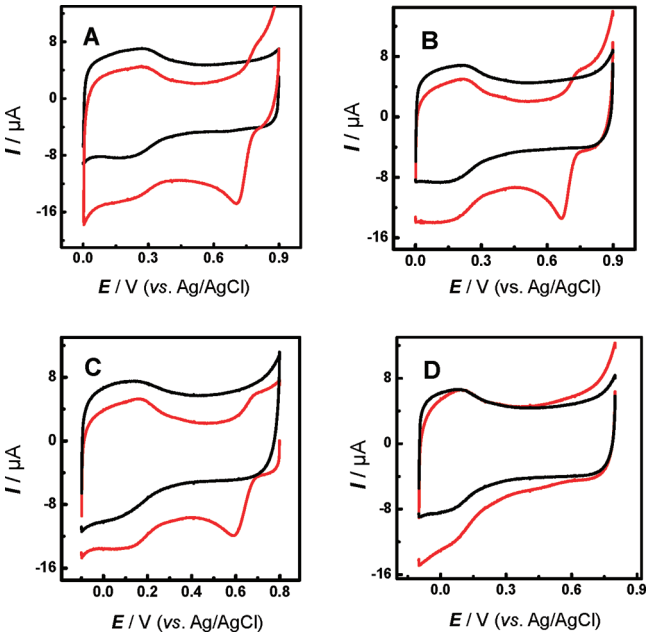

Figure 1. Cyclic voltammograms (CVs) obtained at the laccase/SWNT-modified electrodes in 0.10 M buffers with different pH values: (A) citric acid-buffered solution with pH 4.0; (B) citric acid-buffered solution with pH 5.0; (C) phosphate-buffered solution with pH 6.0; and (D) phosphate-buffered solution with pH 7.2. The buffers were saturated with N_2 (black curves) or ambient air atmosphere (red curves). Scan rate: 10 mV s^{-1}.

process. However, when the laccase/SWNT-modified electrode was immersed in a neutral solution (i.e., phosphate buffer, pH 7.2), no redox peak was observed for the bioelectrocatalytic O_2 reduction within the potential windows employed (Figure 1D). The large difference in the bioelectrocatalytic O_2 reduction in the buffers with different pH values was elucidated with the variations in the catalytic activity of laccase with buffer pH. As reported previously, OH^- can bind to the copper T2/T3 site of laccase in such a way that O_2 cannot be reduced at this site, resulting in a poor catalytic activity of laccase toward O_2 reduction in neutral media.[38]

Because of the unique physical and chemical properties of ionic liquids (ILs), such as negligible vapor pressure, high chemical and thermal stability, and wide potential window, ILs have drawn great attention and have been widely used in various research and industrial fields, such as organic synthesis, electrochemistry, biocatalysis, and chemical engineering over the past two decades.[47−50] In this study, we interestingly found that the overcoating of hydrophobic ionic liquid (i.e., $Bmim^+PF_6^-$) onto the laccase/SWNT-modified electrode eventually enables the bioelectrocatalytic O_2 reduction to take place in 0.10 M phosphate buffer with a neutral pH, as depicted in Figure 2 A. This result essentially indicates that such a procedure well extends the catalytic activity of laccase into neutral media. Furthermore, the onset potential and the peak potential as well as the peak current for the bioelectrocatalytic O_2 reduction remain almost identical in the buffers with pH values of 4.0 and 7.2, presumably suggesting that the bioelectrocatalytic O_2 reduction became pH-independent upon the surface overcoating of the hydrophobic $Bmim^+PF_6^-$ layer.

While more experimental evidence are actually needed to elucidate such phenomenon, the intrinsic properties of

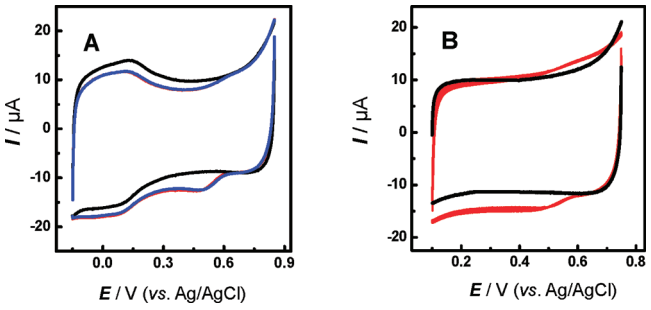

Figure 2. (A) CVs recorded at the $Bmim^+PF_6^-$/laccase/SWNT-modified electrode in 0.10 M buffers with pH values of 4.0 (buffered with citric acid, blue curve) and 7.2 (buffered with phosphate, red curve) under ambient air atmosphere. Black curve represents CV obtained the same electrode in 0.10 M phosphate buffer (pH 7.2) saturated with N_2. Scan rate: 10 mV s^{-1}. The red and blue curves were almost overlapped in the figure. (B) Consecutive CVs obtained at the $Bmim^+PF_6^-$/laccase/SWNT-modified electrode for 50 cycles in 0.10 M phosphate buffer (pH 7.2) saturated with air atmosphere (red curve) or N_2 (blank curve). Scan rate: 10 mV s^{-1}.

$Bmim^+PF_6^-$ may constitute one of the main consequences for the above phenomenon; $Bmim^+PF_6^-$ synthesized by a metathesis reaction between excess HPF_6 and $Bmim^+Cl^-$ was often weakly acidic, even though it was washed with water for several times in the purification process. Moreover, as one kind of aprotic and hydrophobic ionic liquid, $Bmim^+PF_6^-$ may be able to prevent interfacial proton exchange between electrode surface and bulk solution. These properties of $Bmim^+PF_6^-$ actually enable the formation of a weakly acidic layer onto the laccase/SWNT-modified electrodes, in which the catalytic activity of laccase was well retained, even though the electrodes were immersed into the bulk solutions with a neutral pH.

We have also found that the dip-coating of trace amount of phosphate buffer (pH 6.0), prior to the overcoating of $Bmim^+PF_6^-$, remains essential for the observation of the bioelectrocatalytic O_2 reduction at the as-prepared $Bmim^+PF_6^-$/laccase/SWNT-modified electrodes. This was evident from our control experiments with the $Bmim^+PF_6^-$/laccase/SWNT-modified electrodes prepared by dip-coating $Bmim^+PF_6^-$ alone (i.e., without prior dip-coating of 10 μL phosphate buffer) (data not shown). With these electrodes as working electrode, we did not observe the bioelectrocatalytic O_2 reduction under the same conditions as those employed for the $Bmim^+PF_6^-$/laccase/SWNT-modified electrodes with prior dip-coating of trace amount of phosphate buffer (pH 6.0). The phosphate buffer-assisted bioelectrocatalytic O_2 reduction at the $Bmim^+PF_6^-$/laccase/SWNT-modified electrodes in neutral media could be understood by the capability of weakly acidic buffer to prevent laccase being strongly denatured by $Bmim^+PF_6^-$. It has been reported that $Bmim^+PF_6^-$ have a poor biocompatibility with laccase, and the catalytic activity of laccase in $Bmim^+PF_6^-$ was several orders of magnitude lower than that in the aqueous solution.[51,52] To clarify whether the activity of laccase was inhibited or not when it was exposed to the $Bmim^+PF_6^-$ layer overcoated onto the laccase/SWNT-modified electrodes, the activity of laccase was evaluated with an ABTS-based assay based on the catalytic activity of laccase toward the oxidation of $ABTS^{2-}$ into $ABTS^{\bullet-}$ (eq 1).

$$ABTS^{2-} + O_2 \rightarrow ABTS^{\bullet-} + H_2O \quad (1)$$

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As displayed in Figure 3, the aqueous solution of $ABTS^{2-}$ is colorless and has no absorption in visible region (blank curve).

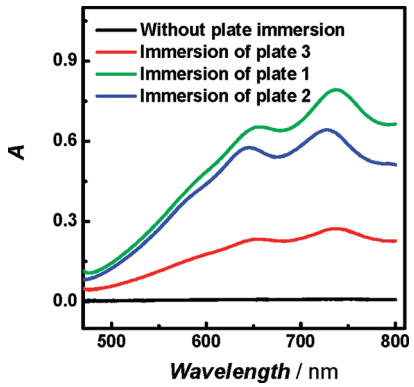

Figure 3. UV–vis spectra of aqueous solution of 0.5 mM ABTS before (black curve) and after (red, blue, and green curves) the immersion of the laccase/SWNT-modified plates into the solution for 5 min. Plate 1 was prepared by simply confining laccase and SWNTs onto the ITO plate. Plate 2 was prepared by consecutively applying aqueous dispersion of SWNTs, laccase solution, phosphate buffer (pH 6.0), and $Bmim^+PF_6^-$ onto the ITO plate. Plate 3 was consecutively applying aqueous dispersion of SWNTs, laccase solution, and $Bmim^+PF_6^-$ onto the ITO plate. Prior to being immersed into the ABTS solution in phosphate buffer, the $Bmim^+PF_6^-$ layer applied onto plates 2 and 3 was removed.

The immersion of plate 1 (prepared by simply confining laccase and SWNTs onto the ITO plate) into the aqueous solution of $ABTS^{2-}$ clearly produces three absorption peaks at ca. 580, 640, and 735 nm in the spectrum (green curve), which were ascribed to the absorption of $ABTS^{\bullet-}$, suggesting that $ABTS^{2-}$ was oxidized into $ABTS^{\bullet-}$ by O_2 under the catalysis of surface-confined laccase through the reaction shown in eq 1. The immersion of plate 2 with the removal of surface-confined $Bmim^+PF_6^-$ layer into the aqueous solution of $ABTS^{2-}$ also produces the absorption peaks in the spectrum (blue curve), but the absorption intensity was slightly lower than that of the solution with immersion of plate 1. This result suggests that the exposure to the $Bmim^+PF_6^-$ layer decreases the activity of laccase, to some extent. However, the immersion of plate 3 with the removal of surface-confined $Bmim^+PF_6^-$ layer into the aqueous solution of $ABTS^{2-}$ produces absorption peaks with largely decreased absorption intensity, as compared with that of the solutions with immersion of plate 1 or 2. These phenomena strongly demonstrate that presence of phosphate buffer between the layers of laccase and $Bmim^+PF_6^-$ essentially preserves the activity of laccase.

The weakly acidic microenvironment formed with $Bmim^+PF_6^-$ overcoating onto the laccase/SWNT-modified electrodes was relatively stable, which could be probed with the consecutive CVs recorded with the electrodes for O_2 reduction in the phosphate buffer (pH 7.2). As demonstrated in Figure 2B, the potential and the peak current for O_2 reduction remain unchanged upon consecutively scanning the electrode for 50 cycles, demonstrating that the as-prepared electrode with $Bmim^+PF_6^-$ overcoating was quite stable for O_2 reduction in neutral media. The unchanged peak current for O_2 reduction also suggests that O_2 could be continuously supplied from the bulk solution during its electrochemical reduction under the catalysis of laccase through a direct electron-transfer mechanism at SWNTs.

Note that, in order to enable laccase-based biocathodes to work in neutral media, Palmore et al. reported an excellent standard molecular biological method to re-engineer the laccase enzyme so that it retains about 50% of its activity at physiological pH.[53] Besides, Serge Cosnier et al. fabricated a three-dimensional structure by mechanical compression of carbon nanotubes and laccase and found that the laccase incorporated into carbon nanotube matrix could preserve its catalytic activity in a neutral solution.[54] Compared with these methods, the strategy demonstrated in this study is technically simple and experimentally durable for bioelectrocatalytic O_2 reduction.

Another universal inhibitor for laccase enzyme is halide ions because of their ability to bind T2 site of laccase with an unusually high affinity, as reported previously.[39] As one kind of halide ion, Cl^- is widely distributed in the biological systems with high concentrations. As expected, as depicted in Figure 4A,

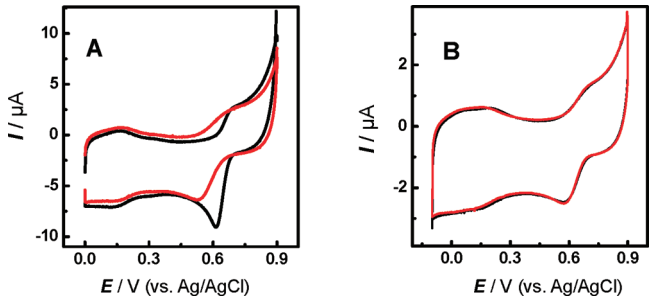

Figure 4. CVs obtained at the laccase/SWNT-modified (A) and the $Bmim^+PF_6^-$/laccase/SWNT-modified (B) GC electrodes in 0.10 M phosphate buffer (pH 6.0) saturated with ambient air in the absence (black curves) and presence (red curves) of 0.15 M KCl. The red and black curves were almost overlapped (B). Scan rate: 2.5 mV s^{-1}.

the presence of Cl^- in the phosphate buffer remarkably leads to the decrease in the peak current and negative shift of the peak potential for the electrocatalytic O_2 reduction at the laccase/SWNT-modified GC electrode. This negative effect of Cl^- on the bioelectrocatalytic O_2 reduction could be understood in terms of the binding ability of Cl^- to the T2/T3 trinuclear copper cluster site, further lowering the catalytic activity for the O_2 reduction at the T2/T3 site.[39] Remarkably, we found that the overcoating of the laccase/SWNT-modified GC electrode with $Bmim^+PF_6^-$ layer well eliminates such a negative effect of Cl^- on the bioelectrocatalytic O_2 reduction, which was evident from the unchanged peak current and peak potential recorded for the O_2 reduction with the presence of Cl^- into the buffer (Figure 4B). The protective effect of $Bmim^+PF_6^-$ overcoating was considered to mainly originate from the hydrophilic property of Cl^- and the hydrophobic feature of $Bmim^+PF_6^-$ overcoating, which actually makes it difficult for Cl^- in the bulk solution to reach the laccase layer through the $Bmim^+PF_6^-$ overcoating.

In addition to its excellent properties to enable the laccase-based biocathode to be active for the bioelectrocatalytic O_2 reduction in neutral media and to eliminate the negative effects of Cl^- on the catalytic activity of laccase toward O_2 reduction, we found that the $Bmim^+PF_6^-$ overcoating well suppresses the electro-oxidation of ascorbic acid on the biocathode. As well documented, AA exists in the biological systems with high concentrations and plays important roles in the biological processes.[55,56] AA possesses a good electrochemical property

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and is readily oxidized at a relatively low potential. While this property facilitates the sensitive electrochemical detection with a high selectivity,[57,58] the easily oxidized feature of AA unfortunately allow it to be readily oxidized at the biocathode employed for O_2 reduction, resulting in dramatic decrease of open circuit potential (OCP) for the BFCs, as typically demonstrated in Figure 5. In 0.10 M phosphate buffers, both

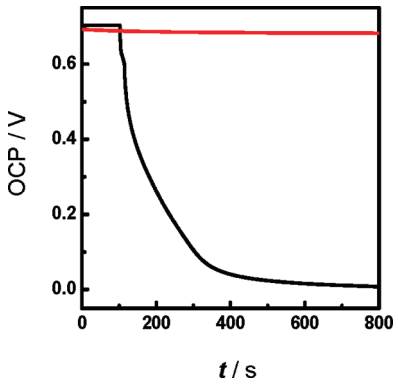

Figure 5. Open circuit potential (OCP)−time curves recorded at the laccase/SWNT-modified electrode (black curve) in 0.10 M phosphate buffer (pH 6.0) and at the $Bmim^+PF_6^-$/laccase/SWNT-modified electrode (red curve) in 0.10 M phosphate buffer (pH 7.2). After the OCP was continuously recorded for ca. 100 s, 0.50 mM AA was added into the buffers. The phosphate buffers were saturated with ambient air.

the laccase/SWNT-modified and the $Bmim^+PF_6^-$/laccase/SWNT-modified electrodes exhibit a good catalytic activity toward O_2 reduction, and the open circuit potential of the biocathodes reaches 0.70 V (vs Ag/AgCl). The striking difference between both biocathodes lies in the changes in the OCP values upon the addition of AA into the buffers: the laccase/SWNT-modified electrode responds quickly with the addition of AA into the phosphate buffer and its OCP value decreases almost to zero (black curve), whereas the OCP value at the $Bmim^+PF_6^-$/laccase/SWNT-modified electrode remains constant (red curve). This difference strongly suggests that the strategy based on the use of $Bmim^+PF_6^-$ overcoating could effectively suppress the AA oxidation at the biocathodes and thereby improves the selectivity for O_2 reduction in the biocathodes.

To clarify the mechanism underlying the suppression of the AA oxidation at the biocathodes through $Bimm^+PF_6^-$ overcoating, we investigated the AA diffusion through the $Bmim^+PF_6^-$ layer with UV−vis spectroscopy. For such a purpose, a U-type tube was used, of which one side was filled with water and the other was filled with the aqueous solution of AA. $Bmim^+PF_6^-$ was filled in the space between water and AA solution (Figure 6 A). The thickness of $Bmim^+PF_6^-$ layer was around 2 cm, and the thickness of water and AA solution layers was about 4 cm. As displayed in Figure 6 B, the adsorption spectra of water and AA solution filled in each side of the U-type tube almost show no change after the U-type tube was filled with water, $Bmim^+PF_6^-$, and AA solution for 1 h, implying that AA does not diffuse through the $Bmim^+PF_6^-$ layer. This result may offer a consequence for the suppression of AA oxidation at the biocathodes with the $Bmim^+PF_6^-$ overcoating, as observed in Figure 5.

In order to demonstrate the validity of our strategy to constructing BFCs that can work in biocompatible media, we

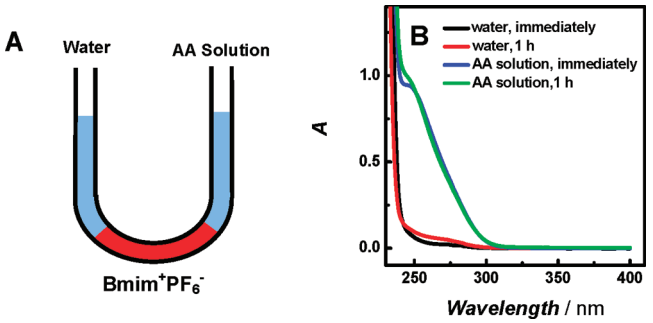

Figure 6. (A) Schematic illustration of a U-type tube filling with water, $Bmim^+PF_6^-$, and AA solution (40 μM). (B) UV−vis absorption spectra of water and AA solution at two sides of the U-type tube containing hydrophobic $Bmim^+PF_6^-$ at the bottom. The spectra were recorded immediately and 1 h after water, AA solution, and $Bmim^+PF_6^-$ layer were filled into the U-type tube, as indicated in the figure.

assembled an AA/O_2 BFC with the as-prepared $Bmim^+PF_6^-$/laccase/SWNT-modified electrode as the biocathode for O_2 reduction and a SWNT-modified electrode as bioanode for AA oxidation. Figure 7 shows the polarization curve and the

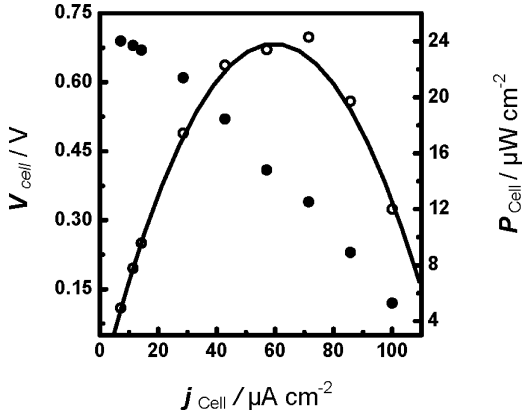

Figure 7. Polarization curve (solid point) and relationship between the power output (P_{cell}) and current density (j_{cell}) (empty point) of the assembled AA/O_2 BFC in 0.10 M quiescent phosphate buffer (pH 7.2) containing 0.50 mM AA under ambient air.

relationship between the power density (P) and the current density (j) of the assembled AA/O_2 BFC in quiescent phosphate buffer (pH 7.2) containing 0.50 mM AA under ambient air. Under these conditions, the open circuit voltage of the cell is ca. 0.73 V (vs Ag/AgCl) and the maximum of the power density reaches 24 μW cm^{-2} at 0.4 V.

4. CONCLUSION

By taking advantage of the intrinsic properties of room temperature ionic liquid $Bmim^+PF_6^-$, we have demonstrated an effective approach to development of new kind of laccase-based biocathodes with improved biocompatibility. The simple surface overcoating of $Bmim^+PF_6^-$ onto the laccase/SWNT-modified electrodes endows the as-prepared laccase-based biocathodes with a good bioelectrocatalytic activity toward O_2 reduction in neutral media and a high tolerance against Cl^-. Moreover, the $Bmim^+PF_6^-$ overcoating applied to the laccase-based biocathodes also well suppresses the oxidation of ascorbic acid at the biocathodes and thus avoids the AA-induced short

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circuit in the laccase-based BFCs. Such properties of the as-prepared biocathodes substantially enable them to work efficiently for the O_2 reduction in physiological solutions (neutral pH, with the presence of chloride ion and acid acid). This study essentially paves a simple avenue to the development of new kinds of BFCs for future biological applications such as in vivo power sources, biotransformation, and biosensor.

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Notes

The authors declare no competing financial interest.

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