

## Co-Electrodeposition of Bilirubin Oxidase with Redox Polymer through Ligand Substitution for Use as an Oxygen Reduction Cathode<sup>†</sup>

Hyosul Shin and Chan Kang\*

Department of Chemistry, Research Institute of Physics and Chemistry, Chonbuk National University, Jeonju, Jeonbuk 561-756, Korea. \*E-mail: chankang@jbnu.ac.kr

Received July 28, 2010, Accepted September 3, 2010

The water soluble redox polymer, poly(*N*-vinylimidazole) complexed with Os(4,4'-dichloro-2,2'-bipyridine)<sub>2</sub>Cl]<sup>+</sup> (PVI-[Os(dCl-bpy)<sub>2</sub>Cl]<sup>+</sup>), was electrodeposited on the surface of a glassy carbon electrode by applying cycles of alternating square wave potentials between 0.2 V (2 s) and 0.7 V (2 s) to the electrode in a solution containing the redox polymer. The coordinating anionic ligand, Cl<sup>-</sup> of the osmium complex, became labile in the reduced state of the complex and was substituted by the imidazole of the PVI chain. The ligand substitution reactions resulted in cross-linking between the PVI chains, which made the redox polymer water insoluble and caused it to be deposited on the electrode surface. The deposited film was still electrically conducting and the continuous electrodeposition of the redox polymer was possible. When cycles of square wave potentials were applied to the electrode in a solution of bilirubin oxidase and the redox polymer, the enzyme was co-electrodeposited with the redox polymer, because the enzymes could be bound to the metal complexes through the ligand exchange reactions. The electrode with the film of the PVI-[Os(dCl-bpy)<sub>2</sub>Cl]<sup>+</sup> redox polymer and the co-electrodeposited bilirubin oxidase was employed for the reduction of O<sub>2</sub> and a large increase of the currents was observed due to the electrocatalytic O<sub>2</sub> reduction with a half wave potential at 0.42 V vs. Ag/AgCl.

**Key Words:** Electrodeposition, Redox polymer, Oxygen reduction, Bilirubin oxidase, Enzyme electrode

### Introduction

The redox polymers have been employed for wiring redox enzymes to conduct electrons between the redox centers of the enzymes and the electrode surface, and, using chemical cross-linkers, efficient electrocatalysts for glucose oxidation and O<sub>2</sub> reduction were formed.<sup>1-6</sup> Multi copper enzymes such as bilirubin oxidase (BOD) and laccase were "wired" to a carbon electrode by redox polymers and the electrocatalytic four-electron reductions of O<sub>2</sub> were reported.<sup>1-4</sup> The reaction centers of laccase from *Coriolus hirsutus* were wired to hydrophilic carbon cloth using poly-*N*-vinylimidazole polymer complexed with [Os(tpy)(dme-bpy)]<sup>2+/3+</sup> (PVI-Os(tpy)(dme-bpy); tpy = terpyridine; dme-bpy = 4,4'-dimethyl-2,2'-bipyridine).<sup>1,2</sup> At this cathode, O<sub>2</sub> was electro-reduced at -0.13 V vs. the reversible potential of the O<sub>2</sub>/H<sub>2</sub>O electrode in pH 5 citrate buffer at 37.5 °C, in the absence of chloride. Bilirubin oxidase from *Myrothecium verrucaria* (*Mv*) or *Trachyderma tsunodae* (*Tt*) was wired to the carbon cloth by the copolymer of polyacrylamide and poly(*N*-vinylimidazole) complexed with [Os(4,4'-dichloro-2,2'-bipyridine)<sub>2</sub>Cl]<sup>+/2+</sup> (PAA-PVI-[Os(dCl-bpy)<sub>2</sub>Cl]<sup>+/2+</sup>).<sup>3,4</sup> At these BOD cathodes, under physiological conditions (pH 7.4, 0.15 M NaCl, 37.5 °C), O<sub>2</sub> was reduced at much smaller overpotentials than that using smooth platinum.<sup>3,4,7</sup> In these redox hydrogel films, the enzymes are covalently bound and their orientation and number of layers are irrelevant.<sup>8</sup>

If the redox polymers satisfy certain conditions, they can be electrodeposited and the enzyme molecules can be co-electrodeposited to form electrocatalyst films for the selective electro-

catalytic reactions of substrates.<sup>9</sup> Enzymes such as glucose oxidase, horseradish peroxidase, soybean peroxidase, and laccase were co-electrodeposited, thereby successfully producing electrocatalyst films.<sup>9</sup> For this purpose, the water soluble redox polymer should contain a ligand bound polymer backbone and transition metal complexes with labile ligands. When the transition metal complexes attached to the polymer chains are reduced, the inner-sphere labile ligands are exchanged by the more strongly coordinating ligands, such as pyridine or imidazole, bound to the polymer chains. This ligand exchange produces cross-linking between the polymer chains, resulting in the formation of an electrodeposited film on the electrode surface. For the redox polymers to be electrodeposited based on ligand substitution, in addition to presence of the labile ligands to be exchanged and free ligands to be coordinated, the surface density of the adsorbed redox polymer must be high, in order for the neighboring chains to exchange ligands and the produced film must be electrically conducting.<sup>9</sup>

In the present study, the redox polymer of poly(*N*-vinylimidazole) complexed with Os(4,4'-dichloro-2,2'-bipyridine)<sub>2</sub>Cl]<sup>+</sup> (PVI-[Os(dCl-bpy)<sub>2</sub>Cl]<sup>+</sup>) was employed. The electrically conducting redox polymer has free imidazole ligands and the osmium complex contains a labile Cl<sup>-</sup> ligand in its inner sphere. We were therefore able to successfully show the electrodeposition of the PVI-[Os(dCl-bpy)<sub>2</sub>Cl]<sup>+</sup> redox polymer on the surface of a glassy carbon (GC) electrode by applying alternating potentials to reduce and oxidize the osmium complexes. When the BOD enzymes with amine groups were present, they were co-electrodeposited with the redox polymer and the prepared electrode was employed as a cathode for the electrocatalytic reduction of O<sub>2</sub>.

<sup>†</sup>This paper is dedicated to Professor Hasuck Kim for his outstanding contribution to electrochemistry and analytical chemistry.

## Experimental

**Chemicals and materials.** Bilirubin oxidase (BOD) from *Trachyderma tsunodae* (Ti) (1.3.3.5, 1.94 u/mg) was purchased from Amano (Lombard, IL). All other chemicals were used as received. The pH 7.4, 20 mM phosphate buffer (PB) and the physiological pH 7.4, 20 mM phosphate, 0.15 M NaCl buffer (PBS) were prepared using deionized water. The powdered BOD enzymes were stored in the refrigerator for the stability and a solution for each measurement was freshly prepared.

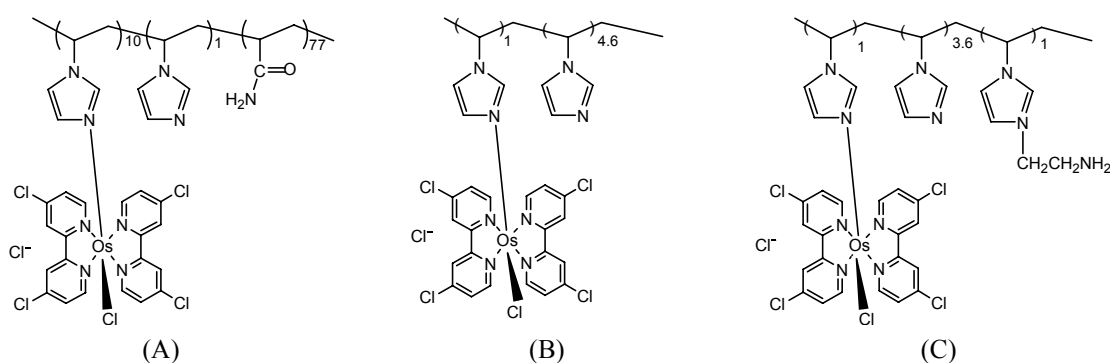
The redox polymer, poly(*N*-vinylimidazole) complexed with  $[\text{Os}(\text{4,4'-dichloro-2,2'-bipyridine})_2\text{Cl}]^+$  (PVI- $[\text{Os}(\text{dCl-bpy})_2\text{Cl}]^+$ ), was synthesized as previously reported.<sup>10</sup> The osmium complex,  $[\text{Os}(\text{4,4'-dichloro-2,2'-bipyridine})_2\text{Cl}_2]$  ( $\text{Os}(\text{dCl-bpy})_2\text{Cl}_2$ ), and the poly vinylimidazole (PVI) were separately synthesized following the literature procedures.<sup>11-16</sup> The imidazoles of the polymer were then coordinated to the osmium complexes. Some free imidazoles of the PVI-Os redox polymer were quaternized with ethylamine group ( $-\text{CH}_2\text{CH}_2\text{NH}_3$ ) to give ethylamine-quaternized redox polymer, EA-PVI- $[\text{Os}(\text{dCl-bpy})_2\text{Cl}]^+$ . The quaternization reaction followed the procedure in the references.<sup>17,18</sup> As analyzed,<sup>10</sup> one Os-complex was attached every 5.6 imidazoles and one of free every 4.6 imidazoles was quaternized with one ethylamine group (Figure 1). The copolymer of polyacrylamide and poly(*N*-vinylimidazole) complexed with  $[\text{Os}(\text{dCl-bpy})_2\text{Cl}]^+$  (PAA-PVI- $[\text{Os}(\text{dCl-bpy})_2\text{Cl}]^+$ ) was synthesized as previously reported.<sup>3</sup> The structures of the redox polymers, PAA-PVI- $[\text{Os}(\text{dCl-bpy})_2\text{Cl}]^+\text{Cl}^-$ , PVI- $[\text{Os}(\text{dCl-bpy})_2\text{Cl}]^+\text{Cl}^-$ , and EA-PVI- $[\text{Os}(\text{dCl-bpy})_2\text{Cl}]^+\text{Cl}^-$ , are shown in Figure 1.

**Apparatus and procedures.** The measurements were performed using a Model CHI 630 potentiostat (CH-Instruments, Austin, TX) controlled through a personal computer. The rotation of the electrode was controlled using a Pine Instrument rotator (Grove, PA). A three electrode cell that had a GC working electrode, an Ag/AgCl (3 M KCl) reference electrode, and an auxiliary platinum wire electrode was used. The cell with a water jacket was kept at 37.5 °C by means of an isothermal circulator. Argon gas pre-saturated with water was used to remove the oxygen from the solution before performing the measurements and was flowed over the solution during the measurements. A GC electrode (3.0 mm diameter, area of 0.071 cm<sup>2</sup>) was polished with 0.3 μm alumina powder, sonicated and rinsed with deionized water. To make the surface hydrophilic, plasma

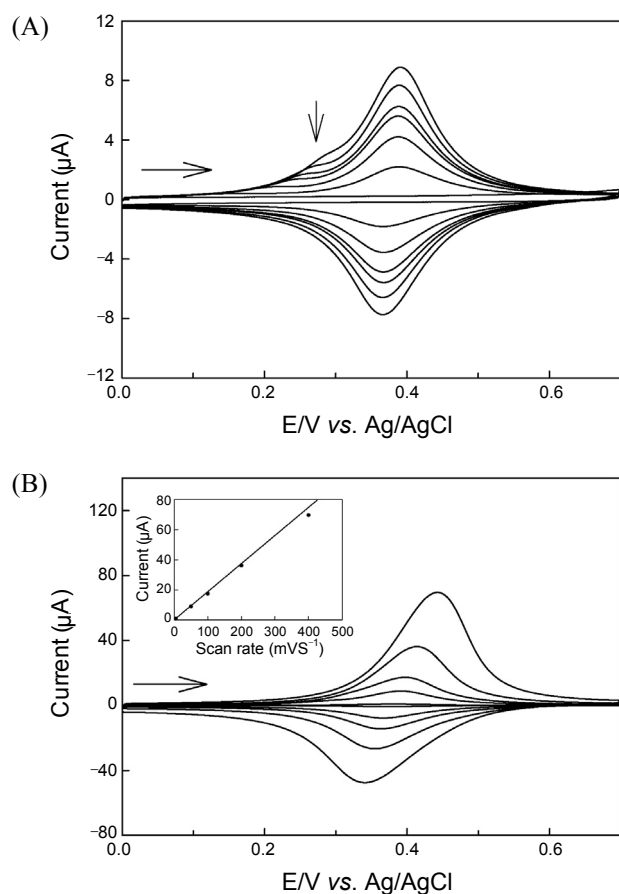
treatment ( $\text{O}_2$ , 1 torr, 5 min) was carried out. For electrodeposition, the potential was stepped from 0.0 V (2 seconds) to 0.7 V (2 seconds) in a solution of the redox polymer (0.3 mg/mL) in pH 7.4 20 mM phosphate buffer at room temperature under argon purging. For the co-electrodeposition of the enzyme, a solution of 0.3 mg/mL redox polymers and 0.3 mg/mL Ti-BOD was used.

## Results and Discussion

**Electrodeposition of redox polymers.** The voltammograms in Figure 2A show the redox waves of the PVI- $[\text{Os}(\text{dCl-bpy})_2\text{Cl}]^{+/2+}$  couple electrodeposited on the GC surface. The GC electrode was initially immersed in a solution of 0.3 mg/mL PVI- $[\text{Os}(\text{dCl-bpy})_2\text{Cl}]^+$  and square wave potentials between 0.7 V and 0.2 V, which are sufficient to completely oxidize and reduce the redox polymer, respectively, were applied for a duration of 2 seconds in each step. The electrode was then transferred to the pure supporting electrolyte and the voltammograms shown in Figure 2A were obtained. For this series of voltammograms, the electrodes subject to 0, 100, 300, 600, 800, 1100, and 1500 cycles of the square wave potentials were employed. Increasing redox waves were measured as the number of the applied cycles increased. The measured formal potential of 0.38 V is the same as the value from the redox peaks of the PVI- $[\text{Os}(\text{dCl-bpy})_2\text{Cl}]^{+/2+}$  couple<sup>10</sup> and it is evident that the redox waves obtained in Figure 2A were due to the adsorbed PVI- $[\text{Os}(\text{dCl-bpy})_2\text{Cl}]^+$  redox polymer. The adsorption of the redox polymer on the electrode can be explained by the electrodeposition of the water soluble redox polymers. The coordinating anionic ligand,  $\text{Cl}^-$  of the osmium complex, becomes labile in the reduced state of the complex because of the decreased coulombic component of the binding energy, and it can be substituted by the imidazole of the PVI chain. The PVI chains were then cross-linked by the ligand substituted osmium complexes, which makes the water soluble redox polymer water insoluble and, consequently, it is deposited on the electrode surface. The surface density of the redox polymer needs to be high enough for the ligand substitution and cross-linking reactions to occur between the neighboring polymer chains. The GC surface was pre-oxidized by plasma treatment to make it hydrophilic. The charged redox polymer may be physically adsorbed on the surface, thereby reducing the distances between the polymer chains. Even in its



**Figure 1.** Structures of (A) PAA-PVI- $[\text{Os}(\text{dCl-bpy})_2\text{Cl}]^+\text{Cl}^-$ , (B) PVI- $[\text{Os}(\text{dCl-bpy})_2\text{Cl}]^+\text{Cl}^-$ , and (C) EA-PVI- $[\text{Os}(\text{dCl-bpy})_2\text{Cl}]^+\text{Cl}^-$  redox polymers.

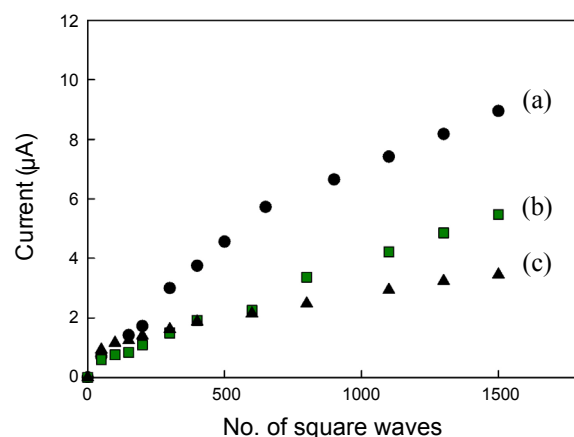


**Figure 2.** (A) Cyclic voltammograms in a pure supporting electrolyte at a GC electrode. Square wave potentials between 0.7 V and 0.2 V with a duration of 2 seconds at each step were previously applied to the electrode in 0.3 mg/mL PVI-[Os(dCl-bpy)<sub>2</sub>Cl]<sup>+</sup> solution, and the electrode was transferred to a pure supporting electrolyte. For the series of voltammograms from the innermost, 0, 100, 300, 600, 800, 1100, and 1500 cycles were applied to the electrode, respectively. Scan rate; 50 mV/sec. (B) Cyclic voltammograms at the electrode with 1500 cycles of square waves applied. The scan rate was controlled as 20, 50, 100, 200, and 400 mV/sec. The inset is a plot of the peak current vs. scan rate. Supporting electrolyte; pH 7.4, 20 mM phosphate buffer at 37.5 °C.

adsorbed state, the deposited film is electrically conducting, as the osmium complexes are mobile enough to conduct electrons though the film layer, resulting in increasing redox waves, as shown in Figure 2A, and the continuous electrodeposition of the redox polymer was observed.

The mixed peaks from the two different osmium complexes, with the Cl<sup>-</sup> ligand exchanged and not-exchanged, make the voltammograms asymmetrical and the currents on the left side a little higher. The small shoulder peaks on the left side (marked by an arrow in Figure 2A) of the main peaks were assigned as those from the osmium complexes in which the Cl<sup>-</sup> ligands were exchanged with imidazoles. The formal potential of the Os<sup>2+/3+</sup> couple with the imidazole ligand was measured to be about 0.27 V, which is more negative than that of the Os<sup>2+/3+</sup> couple with the Cl<sup>-</sup> ligand, and the fraction of the complexes which underwent the ligand exchange was estimated to be approximately 12%.

The electrodeposition process is relatively rapid and, after



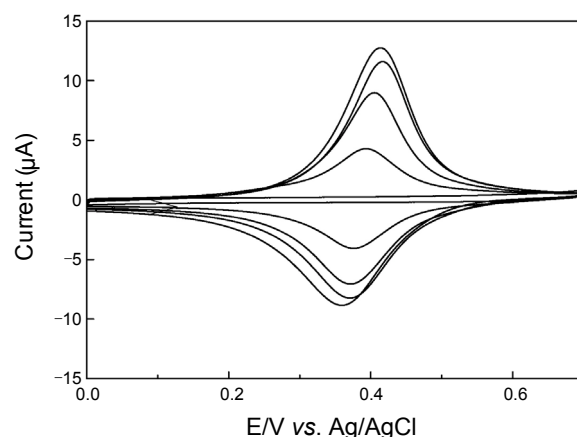
**Figure 3.** Dependence of the electrodeposited amount on the number of square wave cycles for (a) PVI-[Os(dCl-bpy)<sub>2</sub>Cl]<sup>+</sup> (circles), (b) PAA-PVI-[Os(dCl-bpy)<sub>2</sub>Cl]<sup>+</sup> (squares), and (c) EA-PVI-[Os(dCl-bpy)<sub>2</sub>Cl]<sup>+</sup> (triangles). The electrodeposition of the redox polymers and voltammetric measurements followed the same procedure as that in Figure 2A. The anodic peak currents were plotted against the number of applied square wave cycles. The other conditions are the same as those in Figure 2A.

400 s (100 cycles), the surface coverage of the deposited redox polymer was estimated to be  $6.2 \times 10^{-10}$  mol/cm<sup>2</sup> from the integration of the redox waves. The 300 cycles of step potentials gave a value of  $13 \times 10^{-10}$  mol/cm<sup>2</sup>. Figure 2B shows the voltammograms obtained at various scan rates with the PVI-[Os(dCl-bpy)<sub>2</sub>Cl]<sup>+</sup> redox polymer electrodeposited using 1500 cycles of square wave potentials (6000 s). The peak current is proportional to the scan rate in a low scan rate range until about 200 mV s<sup>-1</sup> indicates that the charge transfer in the deposited film is still not diffusion limited in that low scan rate range.<sup>19,20</sup> The separation of the peaks also increased with increasing scan rate, which indicates that the reaction is non-Nernstian,<sup>20</sup> and, at a scan rate of 50 mV s<sup>-1</sup>, it was measured to be 25 mV. For the purpose of comparison, the peak separation of the same redox polymer in a hydrogel film using the cross-linker, poly(ethylene glycol)(400) diglycidyl ether (PEGDGE) was found to be 57 mV,<sup>10</sup> indicating that the charge transfer rate in the electrodeposited film was faster. It is known that the apparent electron diffusion coefficient,  $D_{app}$ , of the redox hydrogels depends on the cross-linking of the segments, which affects the mobility of the tethered redox complexes.<sup>8,21</sup> The faster charge transfer in the electrodeposited film is probably because of the lesser cross-linking brought about by the ligand exchange than that of the hydrogel film made using the PEGDGE cross-linker.<sup>8,21</sup>

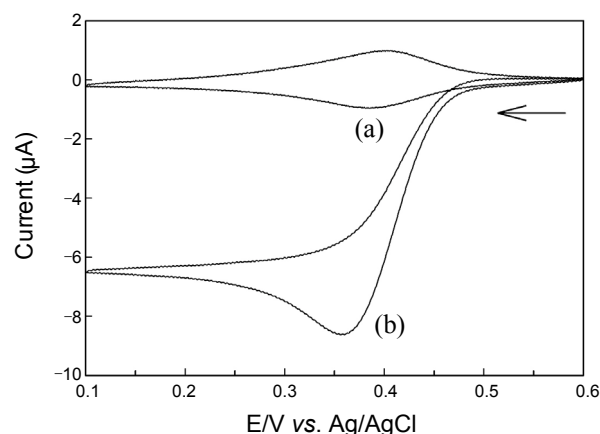
Figure 3a shows the dependence of the size of the voltammetric waves on the number of square waves employed for the electrodeposition of PVI-[Os(dCl-bpy)<sub>2</sub>Cl]<sup>+</sup>. Cycles of square wave potentials were applied to the electrode in a solution of 0.3 mg/mL redox polymer in 20 mM pH 7.4 phosphate buffer. The electrode was then transferred to the pure supporting electrolyte and cyclic voltammograms were measured. The anodic peak current from each voltammogram was plotted against the number of square wave cycles applied and was found to increase continuously until 600 cycles, however, its rate of increase decreased slightly with increasing number of cycles. For the com-

parison, similar plots were also obtained with other redox polymers such as PAA-PVI-[Os(dCl-bpy)<sub>2</sub>Cl]<sup>+</sup> and the ethylamine-quaternized redox polymer, EA-PVI-[Os(dCl-bpy)<sub>2</sub>Cl]<sup>+</sup>. The electrodeposition rate of PAA-PVI-[Os(dCl-bpy)<sub>2</sub>Cl]<sup>+</sup> (squares in Figures 3b) was about 40% slower than that of PVI-[Os(dCl-bpy)<sub>2</sub>Cl]<sup>+</sup> and that of EA-PVI-[Os(dCl-bpy)<sub>2</sub>Cl]<sup>+</sup> (triangles in Figure 3c) was the slowest among these three redox polymers. For PAA-PVI-[Os(dCl-bpy)<sub>2</sub>Cl]<sup>+</sup>, whose structure is shown in Figure 1, the ratio of imidazoles to the osmium complex, in the presence of acryl amide groups, becomes less than that in the PVI-[Os(dCl-bpy)<sub>2</sub>Cl]<sup>+</sup> redox polymer chain, and consequently, the probability of the Cl<sup>−</sup> ligand being exchanged by the imidazole is thought to be much lower. For the other redox polymer quaternized by the ethylamine group, EA-PVI-[Os(dCl-bpy)<sub>2</sub>Cl]<sup>+</sup>, as shown in Figure 1, the imidazole groups are partially quaternized with ethylamine (pK<sub>a</sub> of CH<sub>3</sub>CH<sub>2</sub>NH<sub>3</sub><sup>+</sup>, 10.673),<sup>22</sup> which is protonated at neutral pH, and the Cl<sup>−</sup> ligand exchange by the remaining non-quaternized free imidazoles is thought to be much diminished.

**Co-electrodeposition of redox polymer and enzyme.** The histidine, lysine, and arginine components of proteins are known to act as ligands to transition metal complexes,<sup>23,24</sup> and the exchange of the labile Cl<sup>−</sup> ligand of the osmium complexes in the present redox polymer, PVI-[Os(dCl-bpy)<sub>2</sub>Cl]<sup>+</sup>, by these amino acids is thought to be possible. Therefore, when alternating potentials are applied to the electrode in a solution containing both the enzyme and redox polymer together, the enzymes can also be bound to the metal complexes through ligand exchange reactions and the two species can be co-electrodeposited. For the present system, square wave potentials between 0.7 V and 0.2 V, with a duration of 2 seconds at each step were applied to the electrode immersed in a solution of 0.3 mg/mL PVI-[Os(dCl-bpy)<sub>2</sub>Cl]<sup>+</sup> and 0.3 mg/mL *Tt*-BOD. The concentration of BOD relative to that of the redox polymer was roughly optimized. If the amount of BOD was too much, precipitate was formed.<sup>3,4</sup> If it was too small, the co-electrodeposition of the BOD enzyme became inefficient. The electrode was then washed with deionized water and dried in the air. Cyclic voltammograms were obtained in the pure supporting electrolyte, as shown in Figure 4. For each voltammogram, a different number of cycles of the square wave potentials, as indicated, was applied. Increasing redox waves were measured as the number of applied cycles increased up to 600 cycles, but, as the number of cycles applied was further increased, a reduced increment was measured. The appearance of the redox waves confirms the presence of the electrodeposited film of the redox polymer, which is still electrically conducting. In comparison to the voltammograms of Figure 2A where only the redox polymer film is deposited, different results were observed when the enzymes were presumably co-electrodeposited. First, reduced currents on the left side were measured probably because the Cl<sup>−</sup> ions are exchanged more with the amino acids of the *Tt*-BOD enzymes than with the imidazoles of the polymer chain and the fraction of osmium complexes with the ligand exchange might be less than the case of Figure 2A. The negative surface of the *Tt*-BOD enzyme at pH 7.4<sup>3,4</sup> was readily attracted to the positive osmium complexes and the amino acids of the enzymes replaced the Cl<sup>−</sup> ligands more easily than the neutral imidazoles. Secondly, higher cur-



**Figure 4.** Cyclic voltammograms in a pure supporting electrolyte at a GC electrode. Square wave potentials between 0.7 V and 0.2 V with a duration of 2 seconds at each step were previously applied to the electrode in a solution containing 0.3 mg/mL PVI-[Os(dCl-bpy)<sub>2</sub>Cl]<sup>+</sup> and 0.3 mg/mL *Tt*-BOD. For series of voltammograms from the inner-most, 0, 150, 300, 600, and 900 cycles were applied to the electrode, respectively. The other conditions are the same as those in Figure 2A.



**Figure 5.** Electrocatalytic reduction of O<sub>2</sub> in an O<sub>2</sub>-saturated solution at the electrodeposited BOD cathode (b). The curve (a) was the background voltammogram obtained in the absence of O<sub>2</sub> in the solution. The electrode was prepared by applying 900 square wave cycles as in Figure 4. Scan rate: 5 mV/s. Supporting electrolyte: pH 7.4, 0.15 M NaCl, 20 mM phosphate buffer at 37.5 °C.

rents were measured when the enzymes were present in the solution even with the same number of cycles of applied potentials (under 600 cycles applied). This may be explained by the easier cross-linking with the enzyme molecules than with the imidazoles, resulting in a greater amount of the redox polymer being deposited. However, as the deposited film became thicker, the deposition rate decreased, probably due to the slower electron transfer rate. The decrease of the electron transfer rate is indicated by the larger peak separation of the voltammograms in Figure 4 than those in Figure 2A at the same scan rate.

**Catalytic electro-reduction of O<sub>2</sub> at the electrode with the redox polymer and BOD co-electrodeposited.** If the PVI-[Os(dCl-bpy)<sub>2</sub>Cl]<sup>+</sup> and *Tt*-BOD were really co-electrodeposited to form a film, the electrode could be employed for the electrocatalytic reduction of O<sub>2</sub>. The electrode to which 900 cycles of square

wave potentials were applied, corresponding to the experiment of Figure 4, was used for the reduction of  $O_2$ . Figure 5 shows the cyclic voltammograms in the absence and presence of  $O_2$  (1 atm) (pH 7.4, 20 mM phosphate buffer, 0.15 M NaCl). A large increase of the cathodic currents due to the catalytic  $O_2$  reduction was observed, which indicates that the catalyst material, *Tt*-BOD enzyme, was contained in the deposited film. The film of the redox polymer and co-electrodeposited *Tt*-enzyme has rapid electron-exchanging couples mediating electrons between the enzyme reaction centers and the electrode surface. If a BOD cathode with  $PVI-[Os(dCl-bpy)_2Cl]^+$  prepared by using a chemical cross-linker was used, it was not stable with time because of the continuous ligand exchange in the cathode film showing a changing voltammogram. However, this ligand exchange property can be utilized to form a catalyst film through the electrodeposition. With other redox polymers, such as PAA- $PVI-[Os(dCl-bpy)_2Cl]^+$  and quaternized  $PVI-[Os(dCl-bpy)_2Cl]^+$ , the electrodeposition was much inhibited and BOD catalyst films were prepared by using a chemical cross-linker.<sup>4,10</sup> The half wave potential for the electrocatalytic reduction of  $O_2$  was measured to be 0.42 V vs. Ag/AgCl, which is 40 mV more positive than the value of 0.38 V at the cathode wired with PAA- $PVI-[Os(dCl-bpy)_2Cl]^+$  and similar to the value at the other cathode with the EA-quaternized  $PVI-[Os(dCl-bpy)_2Cl]^+$ . The net catalytic  $O_2$  reduction current was measured as 76  $\mu A$  in Figure 5, which was higher than 50  $\mu A$  and 46  $\mu A$  measured separately with the two other cathodes, respectively, under the same condition. The higher catalytic currents on the cathode prepared by the electrodeposition method may be due to higher electron transfer rate in the deposited film layer than the other redox hydrogel films.

### Conclusions

The redox polymer,  $PVI-[Os(dCl-bpy)_2Cl]^+$ , contains a labile anionic  $Cl^-$  ligand in its inner sphere, which could be substituted with the imidazole ligand of the polymer chains. Hence, when a potential is applied to reduce the osmium complexes, the ligand substitution reaction occurs resulting in the cross-linking of the polymer chains and the electrodeposition of the redox polymer. The other conditions required for the present redox polymer to be electrodeposited are also satisfied. The surface density of the physically adsorbed redox polymer must be high for the neighboring chains to exchange ligands and the deposited film was electrically conducting, thus allowing for continuous electrodeposition. When other redox polymers, PAA- $PVI-[Os(dCl-bpy)_2Cl]^+$  and the ethylamine-quaternized redox polymer, EA- $PVI-[Os(dCl-bpy)_2Cl]^+$ , with fewer imidazoles, and imidazoles partially quaternized causing their coordination to be inhibited, respectively, were employed for the purpose of comparison, much less electrodeposition was observed.

In the present study, *Tt*-BOD enzyme was successfully co-

electrodeposited with the redox polymer,  $PVI-[Os(dCl-bpy)_2Cl]^+$ . In the produced film of the redox polymer and co-electrodeposited *Tt*-enzyme, the electrocatalytic reduction of  $O_2$  occurred at a half wave potential of 0.42 V vs. Ag/AgCl, which is comparable with the results obtained in other redox hydrogel films using a chemical cross-linker.

**Acknowledgments.** This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (KRF-2008-313-C00570). Authors thank to professor A. Heller as this study was derived from the work carried out while the authors were staying in his group at the University of Texas at Austin.

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