Electrodepositing Redox Polymer on Sandwich Complex for the Improvement of Sensitivity in Sandwich Enzyme-linked Immunoassay

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Improvement of the sensitivity of electrochemical sandwich enzyme immunoassay has been achieved by electrodepositing redox polymer on screen-printed carbon electrode surface, on which the sandwich complex was formed.

There has been great interest in the development of sensitive and specific immunoassays for the quantitative determination of analytes of clinical or biological importance.[1] A number of immunoassay systems described in the literature were based on the principle of electrochemical detection of the labeled immunoagent, such as amperometric determination of the product of the reaction catalyzed by the enzyme label in enzyme immunoassay.[2] Recently, a simple method has been developed to electrodeposit redox polymer and co-electrodeposit biological molecule, such as enzyme,[3] avidin,[4] and nucleic acid,[5] on electrode surface. The process involves the exchange of labile, inner-sphere chloride ligand of $Os^{2+/3+}$ complexes of one chain with more strongly coordinated nitrogen ligands of second chain. Amine-containing biomolecules were similarly incorporated into the electrodeposited redox polymer films as the amines also replace inner sphere chloride of the Os^{2+} complexes. And resulting biosensor could be used for enzyme-amplified amperometric sandwich test for RNA and DNA. Completion of the sandwich brings the HRP label into electrical contact with the redox polymer, converting the nonelectrocatalytic base layer into an electrocatalyst for the electroreduction of H_2O_2 to water. Flow of H_2O_2 electroreduction current when the electrode was poised near Ag/AgCl potential indicates the presence of the analyte RNA or DNA. In the present work, we further developed the method into sandwich enzyme immunoassay and improved the sensitivity of system by electrodepositing redox polymer on sandwich complex on screen-printed electrode. The optimization of determination condition using mouse IgG as a model analyte was presented and analytical characteristics of the immunoassay system were evaluated.

The electron-conducting redox polymers, water-soluble poly(vinylimidazole) or copolymer of acrylamide and vinylimidazole, complexed with osmium (4,4′-dimethyl-2,2′-bipyridine) chloride denoted (PVI-Os) and (PAA-PVI-Os), respectively, were prepared by the reported procedures.[6,7] Electrochemical experiments were performed with a CHI 832 potentiostat. A three-electrode system was employed, with a platinum foil counter electrode, a silver/silver chloride reference electrode in saturated potassium chloride (Ag/AgCl) and a screen-printed carbon working electrode. The antibody-modified electrode was prepared in three steps. First, first layer of redox polymer film was electrodeposited from redox polymer (PVI-Os) solutions ($0.65\,mg\,mL^{-1}$) in phosphate buffer (PB, pH 7.1, 20 mM) by applying a steady reducing potential (−1.4 V vs Ag/AgCl) for 60 s. Second, the screen-printed carbon electrode was immersed in phosphate buffer (20 mM, pH 7.1) containing $10\,mg\,mL^{-1}$ goat anti-mouse IgG antibody for 40 min to adsorb the antibody. And then, it was removed, washed with phosphate buffer and electrodeposited to the second layer of redox polymer from redox polymer (PAA-PVI-Os) solutions ($0.65\,mg\,mL^{-1}$) in phosphate buffer (pH 7.1, 20 mM) by applying a steady reducing potential (−1.4 V vs Ag/AgCl) for 40 s. All resulting electrodes were washed with phosphate buffer (pH 7.1) and stored in a refrigerator (4 °C) when not in use.

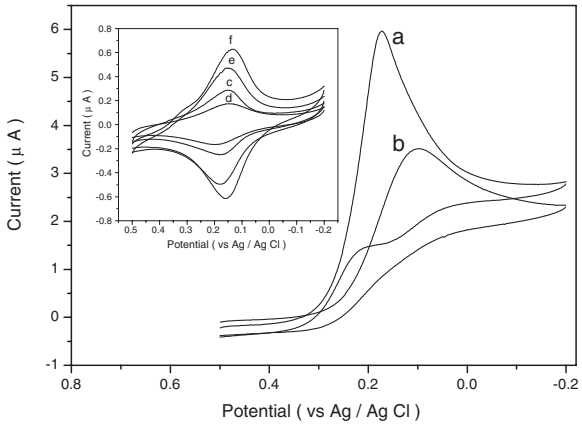

Figure 1. Cyclic voltammograms of the electrode with the electrodeposited redox polymer.

A sandwich scheme of immunoassay was employed. The antibody-modified electrode was immersed in PB (0.1 M, pH 7.4) containing 1% bovine serum albumin (BSA) for blocking, and then incubated with mouse IgG antigen solution (0.1 M PB, pH 7.4, containing 0.15 M NaCl) for 10 min. And then, washing step was performed to remove any unbound antigen. The resulting electrode was immersed in horseradish peroxidase (HRP) labeled conjugate solution (1:100 diluted with 0.1 M PB, pH 7.4, containing 0.15 M NaCl, 1% BSA and 0.05% Tween 20) for 15 min. This represented the second stage of the immuno-interaction and resulted in the formation of the sandwich complex. The electrode was washed thoroughly with PB to remove non-specially bound HRP-conjugated antibodies, which could cause a background response. Last, the electrode containing sandwich complex was immersed in redox polymer (PVI-Os) solutions ($0.65\,mg\,mL^{-1}$) in phosphate buffer (pH 7.1, 20 mM) and apply a steady reducing potential (−1.4 V vs Ag/AgCl) for 60 s to electrodeposite third layer of redox polymer film. After rinsing, the H_2O_2 electroreduction current was measured. A thermostat-

ed bath was used to control the reaction temperature at 25 ± 1 °C.

After each electrodeposition creating a new redox polymer composition film, the cyclic voltammogram (CV) was scanned in PB (pH 7.1, 20 mM) containing 0.15 M NaCl (Figure 1 and inset). Figure 1c showed a typical CV of an electrode with first layer of electrodeposited redox polymer. After the modified electrode was immersed in antibody solution and rinsed, the CV of the electrode was measured and shown in Figure 1d. Immersing in antibody solution decreased the peak heights and increased the separation of the peaks. After electrodeposition of second layer and third layer of redox polymer, CVs were also measured and shown in Figures 1e and 1f (inset), respectively. Well-defined, chemically reversible CV peaks were observed centered at about 150 mV (vs Ag/AgCl), characteristic of the $Os^{2+/3+}$ redox couple; at 5 mV s^{-1} scan rate, the separation of the peaks of the voltammetric electroreduction and electrooxidation waves was less than 20 mV, which imply the redox polymer composition film containing antibody also has rapidly electron-exchanging redox couples. Reduction and oxidation peak currents grew with increasing number of electrodeposition composition films, as shown in Figure 1 (inset, from c, e to f), indicating that a larger amount of redox polymer was brought to the electrode surface. The electrpdeposition of second redox polymer film can further stabilize the adsorbed antibody by coordinative crosslinking between Os presented on the redox polymer and amines of the biomolecule. An additional advantage of using the PAA-PVI-Os instead of the PVI-Os was that they do not bind nonspecific proteins or nucleic acids.[8]

After incubation with antigen (315 pM) and electrodeposition of third layer of redox polymer, a typical voltammogram of an antibody-modified electrode in PB containing 1 mM H_2O_2 was measured and shown in curve a in Figure 1. For comparison, a voltammogram of an antibody-modified electrode without third layer of redox polymer was shown in curve b in Figure 1. Obvious catalytic currents were observed in both cases, which confirm the presence of sandwich complex on the electrode surface. As seen in Figures 1a and 1b, the electrodeposition of third layer of redox polymer improves the electrocatalytic efficiency, resulting in a better communication of the sandwiched peroxidase. Catalytic currents were increased from 3.52 to 5.98 μA. At the same time, electrodeposition of third layer of redox polymer lower detection limits from 19 to 0.63 pM, as shown in Figure 2. When the BSA was added instead of antigen, the change in the reduction current upon adding H_2O_2 was negligible. Apparently, electrodeposition of PAA-PVI-Os redox polymer and immersion in buffer containing BSA decreased non-specific binding the antigen and the HRP-label conjugate on electrode surface.

The magnitude of the electrocatalytic current (i_p) at 0.2 V was chosen as the analytical response. A linear relationship between the current responses and concentration of antigen was obtained and with a dynamic range of 4.4 to 440 pM (0.7 to 70 ng mL^{-1}), as shown in Figure 2. The detection limit was 0.63 pM (0.1 ng mL^{-1}), which is based on the minimum concentration of antigen that gave a signal at least three time larger than

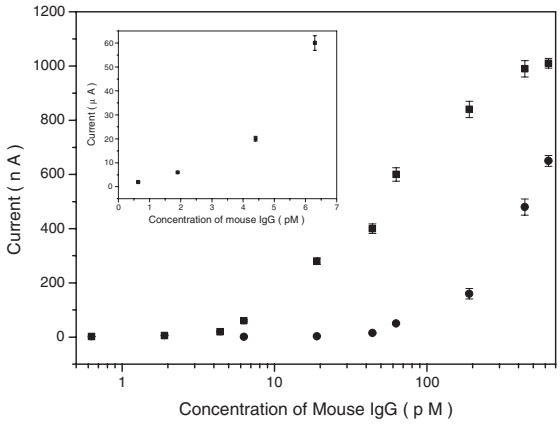

Figure 2. Dependence of the change in current on the concentration of mouse IgG (0.63–630 pM) upon adding 1 mM H_2O_2; (■): biosensor with third layer of redox polymer (Potential: 0.2 V); (●): biosensor without third layer of redox polymer (Potential: 0.1 V).

the signal from the control experiment. The antibody-modified electrode showed good reproducibility for the set of antibody-modified electrode from the same or variant batch, with a standard deviation of less than 4.5 and 6.3%, respectively.

In this work, an improved sensitivity of electrochemical enzyme immunoassay has been achieved by electrodepositing redox polymer on sandwich complex on screen-printed electrode. According to results reported in paper,[9] it might be possible to further improve the sensitivity by optimizing the sensing conditions or using microelectrode. At the same time, this approach was simple, because it does not require enzyme-generated electroactive products.

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