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Simple Electrochemical Immunosensor for the Determination of Rabbit IgG Using Osmium Redox Polymer Films

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Abstract : An amperometric immunosensor for the determination of rabbit IgG is proposed. The immunoassay utilizes a screen-printed carbon electrode on which osmium redox polymer is electrodeposited. This immunoassay detects 0.1 ng/ml of rabbit IgG, which is ~ 10^2 fold higher than the most sensitive enzyme amplified amperometric immunoassay. The assay utilizes a screen-printed carbon electrode which was pre-coated by a co-electrodeposited film of an electron conducting redox hydrogel and a rabbit IgG. The rabbit IgG in the electron conducting film conjugates captures, when present, the anti-rabbit IgG. The captured anti-rabbit-IgG is labeled with horseradish peroxidase (HRP) which catalyzes the two-electron reduction of H_2O_2 to water. Because the redox hydrogel electrically connects HRP reaction centers to the electrode, completion of the sandwich converts the film from non-electrocatalytic to electrocatalytic for the reduction of H_2O_2 to H_2O when the electrode is poised at 200 mV vs. Ag/AgCl.

Keywords : Immunosensor, Electrochemistry, Enzyme-amplification, Hydrogel.

1. Introduction

Enzyme-linked immunosorbent assay (ELISA) is a traditional method for immunoassay.[1-3] Spectroscopic methods are often used for the absorbance of the products following to the enzyme reaction. However, the absorbance detection of the spectroscopic methods is not sensitive enough due to its inherent property. Unlike the spectroscopic immunosensors, electrochemical immunosensors for clinical diagnosis have several advantages such as good selectivity, simple instrumentation, relatively low cost, miniaturization and fully automation.[4-6] However, the electrochemical immunoassay has not been extensively studied till now. Electrochemical immunosensors are basically combined with ELISA method and the catalytic currents are measured by oxidation or reduction of substrates through the enzyme reaction. The amount of labeling enzyme is proportional to the catalytic currents. Mostly labeling enzymes have been reported in the area of the electrochemical nucleic acid sensors. In these assays (a) a capture sequence is co-immobilized with osmium redox hydrogel on an electrode; (b) the modified surface is exposed to the analyzed solution so that, if present, the single-stranded target nucleic acid can be captured; and (c) a yet un-hybridized part of the target nucleic acid can be hybridized with an enzyme-labeled nucleic acid sequence.[7-15] The peroxidases, such as horseradish peroxidase (HRP), thermostable soybean peroxidase, and bilirubin oxidase (BOD) are often used as the labeling enzyme.[16,17] Now the enzyme-labeled surface-bound duplex is detected through an enzyme-catalyzed reaction. Because redox enzymes can be conveniently "wired" through redox hydrogels to electrodes, the presence of the enzyme-labeled duplex can be detected amperometrically through electrooxidation or electroreduction of the substrate of the enzyme.

To "wire" the labeling peroxidase and to cause the electrodes to selectively capture the single stranded target DNA, an electron conducting redox hydrogel was electrodeposited on the electrodes, and then the capture sequence was co-electrodeposited.[18-20]

Heller *et al.* developed an amperometric immunosensor for rabbit IgG using osmium hydrogels on screen-printed carbon electrodes, which were previously modified with avidin and biotin-labeled anti-rabbit IgG.

In the present paper, the attachment of an antibody on the electrode surface was carried out through the antibody-antigen interaction without further modifications on the electrode. The rabbit IgG can be detected through the simple direct immunoassay by two electron electroreduction of H_2O_2 to H_2O, with HRP serving as the label of the immunoassay.

2. Experimental

2.1 Materials and reagents

Horseradish peroxidase (HRP; EC 1.11.1.7, 220 U/mg) was purchased from Toyobo, Japan. Bovine serum albumin (BSA), rabbit IgG (r-IgG), anti-rabbit IgG (anti-r-IgG) and anti-mouse IgG (anti-m-IgG) were purchased from Sigma-aldrich Co. (St. Louis, MO). Monoclonal antibody isotyping kit I (HRP/ABTS) was purchased from Pierce (Rockford, USA). The buffering salts and other chemicals were purchased from

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Sigma-aldrich Co.(St. Louis, MO) and were used as received.

The phosphate buffered saline solution (PBS : 4.3 mM NaH_2PO_4, 15.1 mM Na_2HPO_4, 140 mM NaCl), the washing buffer (4.3 mM NaH_2PO_4, 15.1 mM Na_2HPO_4, 500 mM NaCl and 0.5% Tween 20®), and all other solutions were prepared using deionized water (Barnstead, Nanopure II, Van Nuys, CA).

The electron-conducting redox polymer, PVI-$[Os(bpy)_2Cl]^{+/2+}$, a poly (N-vinylimidazole) (PVI) complexed with $[Os(2, 2'\text{-bipyridine})_2Cl_2]^{2+/3+}$, was synthesized as previously described.[16,20-22]

2.2 Preparation of antibody rabbit IgG labeling with HRP

HRP was activated as following : 5 mg of HRP was dissolved in 1.2 ml deionized water, and then 0.3 ml of 20 mM sodium periodate in 10 mM sodium acetate at pH 5.0 was added. After that solution was incubated at 37°C for 5 ~ 10 min. And then the antibody protein was dissolved to 20 mM sodium carbonate, pH 10.0, and then activated peroxidase, equal or 2 weight (1 : 1 ~ 1 : 2) was added. Finally, HRP conjugating antibody solution was incubated at 4°C for 24hrs (or at 37°C for 2hr), and then the conjugate was purified by dialysis with PBS.

2.3 Instrumentation and electrodes

The electrochemical measurements were carried out with a CH Instrument Model 660A electrochemical detector (CH Instrument Austin, TX), interfaced to a computer. A 0.5 mm diameter platinum wire counter electrode and an Ag/AgCl micro-reference electrode (3 M KCl saturated with AgCl) (Cypress, Lawrence, KS) were used. The working electrodes were 3.0 mm-diameter screen printed disk electrodes (SPEs) described by Dequaire and Heller. They were made by screen-printing hydrophilic carbon ink (Electrodag® 423SS from Acheson, Port Huron, MI) on a flexible polyester film. To confine the hydrophilic carbon ink, a hydrophobic circle was drawn prior to printing the SPEs using DAKO S 2002 felt-tip pen (DAKO Corp., Carpinteria, CA).

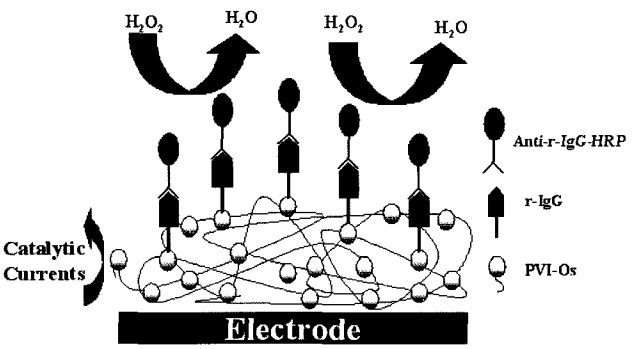
2.4 Electrodeposition of the redox polymer PVI-$[Os(bpy)_2Cl]^{+/2+}$

The earlier described electron-conducting redox polymer PVI-$[Os(bpy)_2Cl]^{+/2+}$, a poly-N-vinylimidazole and the imidazoles complexed with $[Os(bpy)_2Cl]^{+/2+}$ (2, 2'-bipyridine), was co-electrodeposited as follows[16] : 40 μL drops of 1 mg/mL polymer and 1 mg/mL rabbit-IgG solution in 0.43 mM NaH_2PO_4, 1.5 mM Na_2HPO_4, and 14 mM NaCl were pipetted onto the SPEs. The SPEs were poised at −1.4 V vs. Ag/AgCl for 2 minutes, rinsed with deionized water and PBS, and then scanned between 100 mV and 500 mV vs. Ag/AgCl to confirm the electrodeposition of the redox polymer. And then 3 mg/mL BSA in PBS was pipetted onto the SPEs to prevent non-specific absorption, and rinsed with deionized water and washing buffer. The preparation of the electrodes was completed by pipetting onto them 30 μL drops of the rabbit IgG-HRP solution in PBS. The H_2O_2 electroreduction current was measured in 30 μL drop of PBS containing 0.2 mmol/L H_2O_2 at room temperature with the electrode potential poised at 200 mV vs. Ag/AgCl.

3. Results and Discussion

The two steps of the process leading to the electrical "wiring" of HRP when the antibody rabbit-IgG labeling HRP is present in the tested solution are shown in Scheme 1. The electron-conducting redox polymer PVI-$[Os(bpy)_2Cl]^{+/2+}$, a poly-N-vinylimidazole and the imidazoles complexed with $[Os(bpy)_2Cl]^{+/2+}$ (2, 2'-bipyridine), was co-electrodeposited with rabbit-IgG solution onto the SPEs which poised at −1.4 V vs. Ag/AgCl for 2 min. The co-electrodeposition of osmium redox polymers and rabbit-IgG onto SPE is simple, fast and reproducible.

The steady-state cyclic voltammograms of an electrodeposited film PVI-$[Os(bpy)_2Cl]^{+/2+}$ as a function of scan rate are shown in Fig. 1. The cyclic votammograms at slow scan rate (< 50 mV/sec) have quasi-reversible waves. The surface coverage (G) of PVI-$[Os(bpy)_2Cl]^{+/2+}$ is calculated to be 3.0×10^{-10} moles/cm^2,



Scheme 1. Scheme of electrochemical immunoassay using osmium hydrogels.

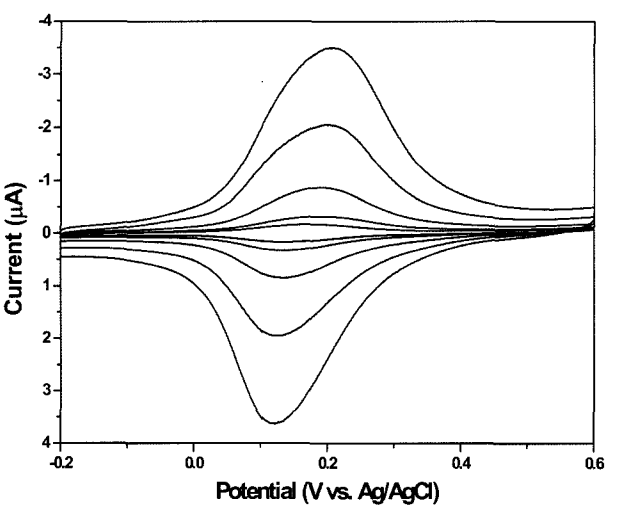
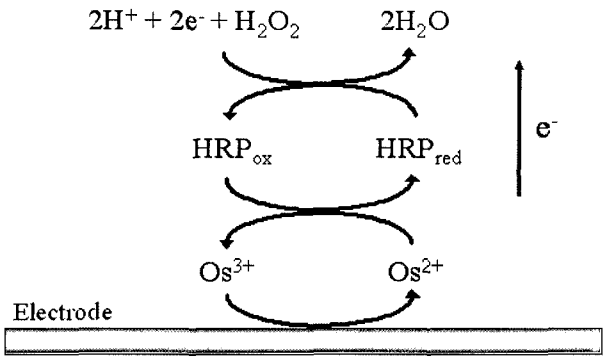
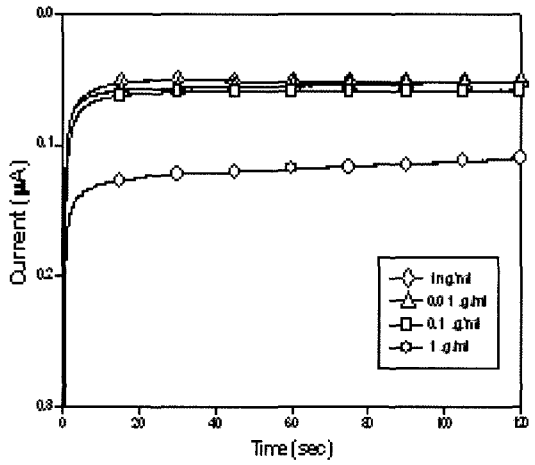


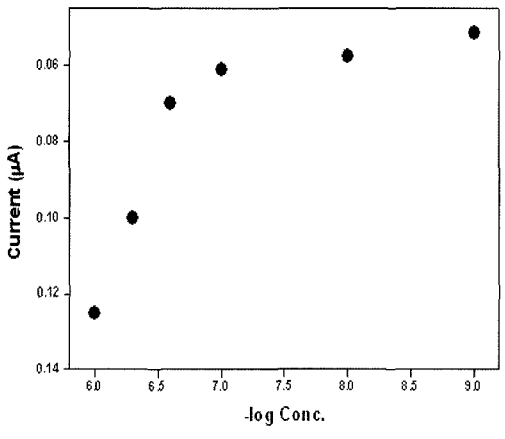
Fig. 1. Cyclic voltammograms of electrodeposited PVI-Os films under air. 3.5 mm-diameter screen-printed carbon electrode; scan rate 10 mV/sec ~ 200 mV/sec; pH 7.2 PBS with 0.14 M NaCl.



Scheme 2. Catalytic electron transfer steps of electrochemical immunoassay using HRP.



(a)



(b)

Fig. 2. Dependence of the H_2O_2 electroreduction current on anti-rabbit-IgG-HRP concentrations. The currents were measured with the 3.0 mm diameter SPEs poised at 0.2 V (Ag/AgCl), PBS buffer, pH 7.2, with 0.14 M NaCl at 25°C in air, 0.2 mmol/L H_2O_2. (a) i-t curves, (b) Plots of catalytic currents at 60 seconds.

which is ~10 fold higher than self-assemble monolayers (SAMs) on electrodes.

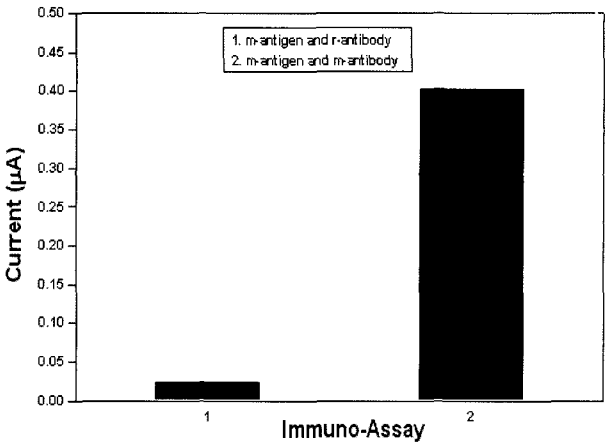


Fig. 3. Cross reactivity of the H_2O_2 electroreduction currents. (1) anti-mouse-IgG-HRP, (2) anti-rabbit-IgG-HRP. The currents were measured with the 3.0 mm diameter SPEs poised at 0.2 V (Ag/AgCl), PBS buffer, pH 7.2, with 0.14 M NaCl and 0.2 mM H_2O_2 at 25°C in air.

Scheme 2 shows the electron transfer steps underlying the catalysis of H_2O_2 electroreduction to water when HRP is electrically contacted with the $Os^{2+/3+}$ complex comprising redox polymer. The electrodeposition and the formation of Os hydrogel are easily confirmed by integrating the area of their voltammograms at slow scan rate (< 10 mV/sec).

Fig. 2 shows the dependence of the H_2O_2 electroreduction currents on the concentration of analyte, antibody rabbit. In the immunoassay, the current increased, with the anti-rabbit-IgG concentration through 1.0 ng/ml ~ 1.0 μg/ml. Anti-rabbit IgG was detected at a concentration of as low as 1.0 ng/ml, 100 times higher than the value reported from the sensitive enzyme amplified amperometric immunoassay which requires complicated modification steps and the reagent-involving chemical reactions. The labeling HRP with the redox hydrogel catalyzes the electroreduction of H_2O_2 to water, in which the concentration of anti-rabbit-IgG-HRP was directly related to increment of the H_2O_2 reduction currents.

The immunoassay with antibody mouse-IgG labeling HRP onto the immobilized rabbit-IgG electrode was used to detect the catalytic electroreduction. Because of the cross reactivity of immunoassay, anti-mouse-IgG-HRP was not perfectly conjugated with rabbit-IgG onto the electrode. The signal was dramatically decreased when antibody mouse-IgG labeling HRP was mismatched with rabbit-IgG.

4. Conclusions

Simply rabbit-IgG can be co-immobilized with Osmium hydrogel polymer onto the electrodes. The bound rabbit-IgG labeling HRP produced a good catalytic current through the electro-conducting films. The catalytic response of the bound rabbit-IgG-HRP increased with increasing the concentrations of rabbit-IgG-HRP. Finally, the electrochemical assay has to show the reasonable evidence of cross reactivity and competitive reaction in order to apply it to the practical clinical diagnosis.

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