A Sensitive Potentiometric Immunosensor Based on Nano-gold/cysteine/nafion-modified Platinum Disk Electrode

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Abstract

A potentiometric immunosensor with the immobilization procedure of antibody (mouse anti-IgG) on nano-Au/Cys/nafion-modified platinum disk electrode has been investigated through nanotechnology, nafion membrane and self-assembled technology. The immunosensor obtained by the immobilization procedure showed lower detection limit of response to antigen (mouse IgG) compared to the current methods, the detection limit is as low as $2 \times 10^{-4}$ ng/mL with a wider linear range from $2 \times 10^{-4}$ to 1 ng/mL. Otherwise, the cyclic voltammetry the optimal conditions for immunosensor’s measurement were also discussed in this article.

Keywords: Potentiometric Immunosensor, Nafion, Colloidal Au

1. Introduction

Potentiometric immunosensors have attracted growing attention with high sensitivity, label-free, small size, and ease in use. The immobilization of biomolecules or the design technology of sensing interface is one of the key factors for immosensors [1]. Generally, direct immobilization of proteins onto bare metal surfaces (such as platinum, gold and silver) can frequently result in their denaturation and bioactivity loss of antibody or antigen. Many new immobilized means [2-8] for proteins (such as enzyme, antibody and antigen) have reported in recent years, and the most popular method for immobilization built up through nano-technology [9-12]. Colloidal particles have a number of attractive properties for the purpose of surface nano-fabrication. Their ability of self-organizing can be utilized to form ordered structures, and the parallel nature of the assembly process makes it possible to produce large numbers of nano-sized features and/or cover large surface areas. High biological compatibility of nanoparticles was another advantage for protein immobilization.

Nafion, highly stable membrane, has found many fields of application including dialysis, osmosis, and electrochemical reactors due to its chemical, thermal and mechanical properties [13-16]. Recently, some papers have reported on application of nafion in amperometric sensors for analytes [17-19] to enhance the stability of protein film. Otherwise, the self-assembled technology [20] was a usual method to immobilize immunoaffinity on the metal surface of transducers and the self-assembled monolayer of thiolate compounds has been widely used.

In this paper, a potentiometric immunosensor was constructed by immobilizing antibody (mouse anti-IgG) on a nano-Au/cysteine/nafion modified platinum disk electrode through nafion membrane, L-cysteine and Au colloids by electrostatic interactions. The successful immobilization procedure was proved by Cyclic voltammograms. The results of immunosensor showed lower detection limit of response to antigen (mouse IgG) compared with the current methods, the detection limit is as low as $2 \times 10^{-4}$ ng/mL with a wider linear range from $2 \times 10^{-4}$ to 1 ng/mL. The optimal work environments of immunosensor were also discussed in this paper.

2. Experimental

2.1 Apparatus and Reagent

Goat-anti-mouse IgG (anti-IgG, 14 mg/ml) and mouse IgG (IgG, 27 mg/ml), 4-mercapto benzoic acid (4-MBA) was purchased were purchased from Yingjun Bioengineering Company (shanghai, China).
Gold chloride was purchased from Shenyang Reagent Factory (China). All other reagents were prepared with chemicals of analytical grade.

Electrochemical workstation (CHI660D) was used to measure the potential change of the immunosensor before and after the antigen-antibody immunoreaction and test cyclic voltammetric characteristics in ferricyanide phosphate buffer solution. A three-compartment electrochemical cell contained a platinum auxiliary electrode, a saturated calomel reference electrode (SCE) and a modified platinum disk electrode as working electrode. Ultrapure water (18.3MΩ) produced by a Human LIP900 system (Human, Korea) was used throughout the experiment. The size of gold nanoparticles was estimated from transmission electron microscopy (H600, Hitachi Instrument Co., Japan).

2.2 Preparation of colloidal Au solution

The 20 nm Au colloid was prepared according to the literature [18]. Solution A: 1mL of 1% HAuCl₄ solution was added to 99mL Ultrapure water. Solution B: 4mL of 1% trisodium citrate solution. Solution A was heated up to boiling as stirring, then solution B was added to solution A immediately. The mixture was heated for 30 min sequentially. The solution color was claret. Gold nanoparticle was investigated by UV-visible spectroscopy (Fig. 1) and the size of prepared gold nanoparticles was confirmed by transmission electron microscopy (see Fig. 2).

2.3 Immunosensor modification

Scheme 1 showed the assembled steps of the immunosensor. The platinum disk working electrode was first immersed in piranha solution (H₂O₂: H₂SO₄ 1:3) for 15 min, then ultrasonicated in nitric acid (1:1), ethanol and water for 10 min, respectively. The cleaned platinum disk electrode was immersed in the nafion methanol solution (v/v, 2%) for 5 min at room temperature and then removed to parch with an infrared light for 20 min. The resulting electrode was thoroughly rinsed with water. Following that, the nafion-modified platinum disk electrode was saturated in L-cysteine (Cys, 20 mM in ultrapure water) and Au colloids solution for 24 h, respectively. Finally, the Au colloid-modified electrode was incubated in anti-IgG solution (20 ng/mL in 0.01M acetate buffer solution, pH 5.5) at 30 °C for 2 h. The immunosensor was prepared for use.
2.4 Potentiometric response of immunosensor

Antibody and antigen have ability for the selective molecular recognition of a target analyte from a complex mixture, and the antigen-antibody interaction has been widely used as the molecular recognition part of immunosensor due to its highly specific interaction. The detection is based on the change in the potentiometric response before and after antigen-antibody reaction. The potentiometric response of the immunosensor towards antigen-antibody is evaluated as the equation:

\[ \Delta E = E_2 - E_1 \]

Where \( E_1 \) is the value of steady-state potentiometric response (versus Ag/AgCl electrode) in a buffer solution before the antigen-antibody reaction, and \( E_2 \) represents the value of the steady-state potentiometric response (versus Ag/AgCl electrode) after antigen-antibody reaction under the same conditions.

In this study, the modified immunosensor was tested as above statements.

3. Results and discussion

3.1 Cyclic voltammetry characteristics of immunosensor

The cyclic voltammetry of ferricyanide is a valuable and convenient tool to monitor the barrier of the modified electrode. Therefore, it was chosen as a marker to investigate the changes of the electrode behavior after each assembly step.

Figure 3 shows cyclic voltammograms (CVs) of differently modified electrodes in a 10mM ferricyanide phosphate buffer solution. Well-defined CVs, characteristic of a diffusion-limited redox process, are observed at the bare platinum disk electrode (Fig. 3a.). After dipping the electrode in the nafion solution, the redox peak disappeared (Fig. 3c). The reason is that the nafion can act as an inert electron and mass transfer blocking layer, and it hinders the diffusion of ferricyanide toward the electrode surface. When cysteine was chemisorbed on the electrode, a remarkable current increase was observed (Fig. 3b.) due to the fact that cysteine acts as a so-called promoter in the electron transfer procedure. After gold nanoparticles were self-assembled on the cysteine-modified electrode, the current remarkably increased (Fig. 3b.), for the nanometer-sized Au colloid immobilized on the surface acts like an electron-conducting tunnel, making it easier for electron transfer to take place. When anti-IgG molecules were adsorbed on the electrode surface, a remarkable current decrease was observed (Fig. 3d.) because the anti-IgG biomacromolecules hinders the diffusion of ferricyanide toward the electrode surface again. CV results confirmed that nafion, Au colloids and anti-IgG were successfully assembled on the platinum disk surface.
3.2 Detection for Antigen

The potentiometric immunosensor has been studied for the detection of antigen-antibody interaction. By injection of antigen (mouse IgG), the response with antibody (mouse anti-IgG) can be tested by potential signal.

Fig. 4 indicates the potentiometric response of anti-IgG/nano-Au/Cys/nafion-modified platinum disk electrode. The electrode exhibits the linear dependence on the logarithm of IgG and potential changes. The linear regression equation was $\Delta E = 4.5331 \log [\text{IgG}] + 17.085$ with a correlation coefficient of 0.9947 and the concentration of IgG is from $2 \times 10^{-4}$ to 1 ng/mL with a detection limit of $2 \times 10^{-5}$ ng/mL. The detection limit of this method is lower at least one quantitative grade than the other current immunosensors.
3.3 Optimization of experimental conditions and characteristics of immunosensor

3.3.1 pH for immunoreaction

The effect of pH is important to the immunosensor behavior, for antigen and antibody are proteins that have their own isoelectric point and colloid Au also has its own pH restriction. pH was studied from 5.0 to 8.5 in PBS (Fig.5). The immunosensor potentiometric response increases with increasing pH value from 5.0 to 7.0 and decreases as the pH increases further. The experimental results showed that the maximum response occurs at pH 7.0 where most immunoreactions exhibit optimal binding. Therefore, a pH 7.0 of PBS was used as the medium for the immunoreaction.

3.3.2 The size of Au colloids for immobilization

The size of the Au nanoparticles plays an important role in the immunosensor behavior. Doron et al. [21] confirmed that the larger sized Au colloids can discontinuously assemble and smaller sized Au
colloids may generate continuous arrays of particles on the base monolayer. However, the very smaller gold nanoparticles, the stronger coulomb repulsion effect among charges. The reason is that the gold nanoparticles are negatively charged species as a result of the adsorption of citrate ions in the immobilization process [22]. Fig.6 showed the potentiometric responses of the immunosensor with different size of colloidal gold among 10, 15, 20, 30 and 40 nm for original immobilization on panar gold electrode, the result showed the immunosensor fabricated with about 20 nm gold nanoparticles exhibits a larger response than other sizes, therefore, 20 nm gold nanoparticles was chosen as the immobilized matrix.

3.3.3 Temperature for immunoreaction

Temperature is vital to the immobilization of antibody because antibody will lose its bioactivity on high temperature value and inhibited its bioactivity on low temperature. The effect of temperature were studied from 15°C to 40°C. Fig.7 showed the potentiometric response characteristic of the immunosensor at different immobilization temperature, results showed at 30°C the immobilization exhibit optimal binding. Then, 30°C was chosen for antibody immobilization through the whole experiment.

3.3.4 Response time of immunosensor

Time is a standard for discerning the capability of immunosensor. One of the original aims of a good immunosensor is detecting samples fleetly. Fig.8 is the potentiometric response time of a certain concentration of antigen. After 10 minutes, the potentiometric response has almost finished. This result can prove the immunoreaction on the novel immunosensor can be detected in a short time because of high electric characteristics of gold nanoparticles.

4. The preliminary detection of actual samples

Blood of mouse (3 months old, Dalian, China) was mixed with trisodium citrate solution (0.1 mg/mL) according to the ratio 1:0.9 after the mouse blood was gained, the obtained blood samples were then placed in refrigerator for 24h at 4°C. The solution was finally divided into two layers automatically and the upper layer, blood serum, was needed in detecting IgG during the experiments.

The samples were detected by the resulting immunosensor for five times. The parallel operating steps were shown as followed: 2 μL blood serum was added into the buffer solution after the freshly fabricated immunosensor which was steady in the solution. And the potential will steady again following the immunoreaction. The potentiometric response (the potential change ΔE) was then obtained. The results which were calculated in the same equation: ΔE= 4.5331 [IgG] + 17.085 were...
listed in Table 1. It was proved that the resulting immunosensor can detect IgG in mouse blood serum and the results were centralized in certain range. The primary results showed the resulting immunosensor could supply a feasible method for detection.

<table>
<thead>
<tr>
<th>Added volume (μL)</th>
<th>Potential change ΔE (mV)</th>
<th>Results (mg/mL)</th>
<th>RSD (%)</th>
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<tr>
<td>2</td>
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<tr>
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5. Method Validation
In this section, horseradish peroxidase were immobilized through the same method and satisfied results which were shown in Fig.9. Fig. 9 indicated the potentiometric response of horseradish peroxidase/nano-Au/Cys/nafion-modified platinum disk electrode. The electrode exhibited the linear dependence on the logarithm of H2O2 and potential changes. The linear regression equation was ΔE=23.46 [H2O2] + 11.182 with a correlation coefficient of 0.9921 and the concentration of H2O2 was from 2×10⁻¹ to 10 ng/mL with a detection limit of 2×10⁻¹ ng/mL.

Figure 4. Calibration curves for the peroxidase/nano-Au/Cys/nafion-modified immunosensor in phosphate buffer solution (0.01 M, pH=7.4) in the presence of different concentrations of antigen.

There was an interesting result that the potentiometric response of horseradish peroxidase was higher than anti-IgG, the mainly reason was that the horseradish peroxidase had better conductive ability than anti-IgG though these two moleculars were both large proteins. However, the sensitivity and the detection limit of anti-IgG were higher than horseradish peroxidase.

6. Conclusion
A potentiometric immunosensor based on nano-Au/Cys/nafion-modified platinum disk electrode is confirmed. The simple manipulations of construction of modified electrode, label-free, stable and improved detection limit are main features of proposed immunosensor. And the results of optimal
measurements showed the immunosensor can work under mild conditions. The further studies are in progress. The method is a good means to immobilize the large proteins in trace analysis and can hopefully contribute to significance on practical application on medical testing and signal processing in future.

7. References
