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Talanta 72 (2007) 1800–1804
Talanta
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A sensitive immunosensor using colloidal gold as electrochemical label

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Received 3 November 2006; received in revised form 6 February 2007; accepted 11 February 2007
Available online 21 February 2007

Abstract

A sensitive immunosensor using colloidal gold as electrochemical label is described. In this method, the capture protein was first immobilized on a carbon paste electrode surface through passive adsorption to bind quantitatively with corresponding antigen and colloidal gold labeled antibody to perform a sandwich assay. To detect the amount of the colloidal gold captured on the electrode surface, the colloid was first oxidized electrochemically to produce $AuCl_4^-$ ions which were adsorbed strongly on the electrode surface. Adsorptive voltammetry was then employed for the determination of the adsorbed $AuCl_4^-$ ions. A linear relationship between reduction wave peak current and the antigen concentration (human IgG) from 10 to 500 ng/ml is obtained with a detection limit of 4.0 ng/ml.

Keywords: Immunosensor; Colloidal gold; Electrochemical label; Adsorptive voltammetry

1. Introduction

In recent years, many studies have reported the application of colloidal gold as label for immunoassay and DNA analysis. Based on its unique electron-opaque properties, the first application of colloidal gold as TEM marker was described in 1971 [1], the method was then introduced for SEM in 1975 [2]. Colloidal gold has also been employed to enhance the SPR or QCM responses [3–8]. These methods do not need the dissolving of the colloidal gold. Some instruments involved are expensive and require skillful personnel to operate. Many other methods, such as inductively coupled plasma mass spectrometry [9], chemiluminescence [10], electrochemical stripping technique [11,12] have also been reported to measure the amount of colloidal gold in immunoassay or DNA assay. All of these methods require dissolving the gold nanoparticles using bromine or other oxidants before determination, such a procedure prolongs the analytical time. Gonzalez-Garcia and co-workers have studied in detail the adsorptive electrochemical behavior of colloidal gold on carbon paste electrode (CPE) in hydrochloric media, based on the reduction process of $AuCl_4^-$ ions produced by electrochemical oxidation of colloidal gold [13]. Such a methodology seems promising for direct determination of gold nanoparticles in electrochemical metalloimmunoassay using colloidal gold as the label. To date only the reaction between streptavidin and biotin was investigated by this method [14].

As we know, the binding force between streptavidin and biotin is strong ($K_c = 10^{15}$) and these two substances are of relatively small size compared with other proteins. To investigate the possible extension of the method to detect other large proteins with relatively weak binding force, in this paper, we apply this methodology to the determination of a normal model antigen, human IgG. The corresponding antibody was first immobilized onto a carbon paste electrode through passive adsorption. After the sandwich immunocomplex was formed, the colloidal gold on the electrode was detected by oxidizing them electrochemically

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doi:10.1016/j.talanta.2007.02.020

to generate $AuCl_4^-$ ions adsorbed strongly on the electrode surface. Compared with electrochemical stripping technique, this method does not need dissolve the gold nanaoparticles using bromine or other oxidants and any electrochemical preconcentration process.

2. Experimental

2.1. Materials and reagents

Goat anti-human IgG antibody, human IgG (10.0 mg/ml), and bovine serum albumin (BSA) were purchased from Beijing Dingguo Biotechnology Development Center (Beijing). Chloroauric acid ($HAuCl_4$) and trisodium citrate were obtained from Shanghai Chemical Reagents (Shanghai, China). Buffers used in this study included 0.05 M sodium phosphate-buffered saline (PBS, pH 7.4) as incubating and washing buffer. Other reagents were of analytical purity, and doubly distilled water was used throughout all the experiments.

2.2. Apparatus

Electrochemical measurements were performed with a three-electrode system comprising a platinum foil as auxiliary electrode, a saturated calomel electrode (SCE) as reference electrode, and the carbon paste electrode (CPE) as working electrode. All electrochemical experiments were implemented on CHI660 electrochemical workstation (Shanghai Chenhua Instruments, Shanghai). UV–vis measurements were carried out on MultiSpec-1501 UV–vis spectrometer (Shimadzu Corporation, Japan) equipped with Hyper UV software.

2.3. Au colloid preparation

Colloidal gold particles with average diameter of approximately 20 nm were prepared according to Natan and co-workers [15] with slight modifications. All glassware used in this preparation was thoroughly cleaned in aqua regia (three parts HCl, one part HNO_3), rinsed in doubly distilled water, and oven-dried prior to use. In a 500 ml round-bottom flask, 250 ml of 0.01% $HAuCl_4$ in doubly distilled water was brought to boil with gentle stirring. To this solution 3.75 ml of 1% trisodium citrate was added. The solution turned deep blue within 20 s and the final color changed to wine-red 60 s later. Boiling was pursued for an additional 10 min, then the heating source was removed, and the colloid was stirred for another 15 min. The colloidal gold solution was stored in a dark bottle at 4 °C and used to prepare antibody-colloidal gold conjugate as soon as possible. The resulting gold nanoparticles were characterized by transmission electron microscopy (TEM) that indicated the particle size of colloidal gold was about 20 ± 3.2 nm and UV–vis spectrometer that showed an absorption peak at 525 nm.

2.4. Preparation of the antibody-colloidal gold conjugate

The antibody-colloidal gold conjugate was prepared by adding 0.6 ml of goat anti-human IgG antibody (1.0 mg/ml) to 10 ml of pH-adjusted colloidal gold solution (pH 9.0), followed by incubating at room temperature for 1 h, during which the antibody adsorbed onto the gold nanoparticles through a combination of ionic and hydrophobic interactions. The conjugate was then centrifuged at $18{,}500 \times g$ for 20 min. Two phases were obtained: a clear to pink supernatant of unbound antibody and a dark red, loosely packed sediment of the antibody-labeled immunogold. The supernatant solution was discarded and the soft sediment of immunogold was rinsed by 10 ml of 1% BSA (dissolved in 0.05 M PBS) and collected after a second centrifugation at $16{,}000 \times g$ for 20 min. Finally, the conjugate was resuspended in 10 ml of 1% BSA added to increase stability of immunogold colloid and minimize the non-specific adsorption during the assays. Conjugates can be stored at 4 °C for more than 1 month without loss of activity.

2.5. Fabrication of the carbon paste electrodes

The solid carbon paste electrodes were prepared by adding graphite (1.0 g) to melted paraffin (1.0 g), and the mixture was blended thoroughly to obtain a homogeneous paste. The resulting mixture was used to fill into a Teflon tube (6 mm inner diameter and 10 mm deep) and pressed tightly. A carbon rod was inserted into the tube to achieve electrical contact. The carbon paste electrodes were polished on a sheet of printing paper.

2.6. Electrochemical pretreatment of the carbon paste electrodes and adsorption of the antibody

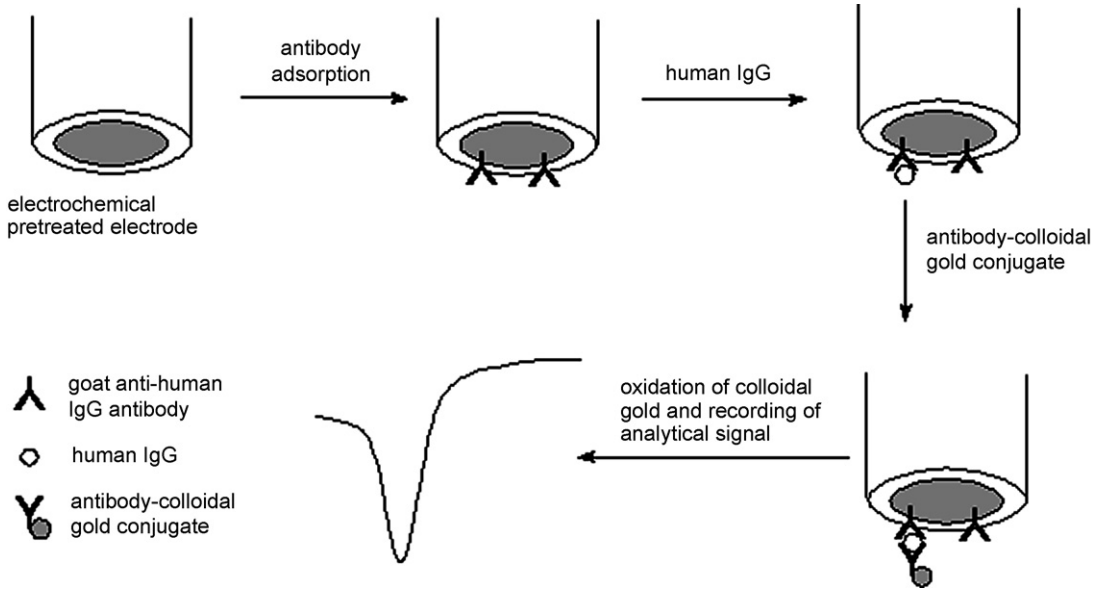
Our previous work showed that electrochemically pretreated electrode surface would enhance protein adsorption [16]. So before antibody adsorption, the carbon paste electrode was pretreated in 0.1 M sodium hydroxide solution with a potential of 2.0 V versus SCE for 10 min, the electrode was then rinsed with 0.05 M PBS. The modification of the carbon paste electrode with antibody was accomplished by coating 50 μl of goat anti-human IgG antibody solution (pH 7.4) on the electrode surface. After 1 h, the antibody-modified electrode was washed with PBS for 8 times. Then 50 μl of 1% BSA was introduced to block the residue-free site for 1 h to form the immunosensor.

2.7. Immunoassay procedure

As shown in Scheme 1, a sandwich immunoassay procedure was adopted for the determination of human IgG. The immunosensor was first incubated in different concentration human IgG solution for 1 h at 37 °C, and then in antibody-colloidal gold conjugate solution for another 1 h. After thorough rinsing with PBS solution to remove any unbound tracer, the electrode was placed in an electrochemical cell containing 10 ml of 0.1 M HCl solution to monitor the amount of colloidal gold on the electrode surface.

2.8. Gold oxidation and signal recording

A potential of 1.30 V was applied to the immunosensor for 150 s. Then a potential scan was performed from 1.30 to 0 V at



Scheme 1. The immunoassay procedure.

a scan rate of 100 mV/s. The analytical procedure is based on the following electrode process: the oxidation of the colloidal gold producing $AuCl_4^-$ ions absorbed on carbon paste electrode surface:

$$Au_{colloid(ads)} + 4Cl^- \rightarrow AuCl_4^-{}_{(ads)} + 3e^-$$

Then $AuCl_4^-$ ions were electrochemically reduced at 0.43 V in 0.1 M HCl solution generating a reduction wave:

$$AuCl_4^-{}_{(ads)} + 3e^- \rightarrow Au_{(ads)} + 4Cl^-$$

3. Results and discussion

3.1. Optimization of the experimental conditions

3.1.1. Selection of the amount of coating antibody on colloidal gold

Since the whole immunoassay was based on the interaction of the goat anti-human IgG antibody and colloidal gold through ionic and hydrophobic bond, the amount of coating antibody on colloidal gold, which was one of the most important components of this immunosensor, should be optimized. A UV–vis absorption curve for the antibody-colloidal gold conjugate was constructed to determine the amount of antibody necessary to coat the exterior of the gold nanoparticles [4]. The solutions were prepared by adding goat anti-human IgG antibody (1 mg/ml) from 10 μl in 10-μl increments into the cuvettes containing 1.0 ml of 20-nm diameter colloidal gold solution adjusted to pH 9.0 using K_2CO_3. The volumes of these samples were corrected to 1.150 ml with doubly distilled water and 100 μl of 10% NaCl solution was added to each. Then the solutions were agitated for 10 min. After that, these samples were recorded at 525 nm, the absorption peak of original colloidal gold, and plotted versus the amount of coating protein. The amount of coating protein where the increase of absorbance starts to be insignificant was taken as optimum for the reason that the less aggregation of the conjugates would result in less red shift, slowing down the increase of the absorbance at 525 nm. As shown in Fig. 1, the increase of absorbance along with the increase of the antibody volume inclines to be stable over 50 μl. Therefore, the optimum amount of goat anti-human IgG for coating the 20-nm gold nanoparticles is 60 μl per 1 ml colloidal gold solution, which is effective to prevent aggregation.

3.1.2. Selection of the oxidation potential

Oxidation potential has much effect on the reduction wave peak current of $AuCl_4^-$ ions which were produced by electrochemical oxidation of colloidal gold. The oxidation potential was tested over a potential range from 1.25 to 1.40 V. After incubated with 10 μg/ml human IgG and antibody-colloidal gold conjugate solution, the immunosensor was placed in an

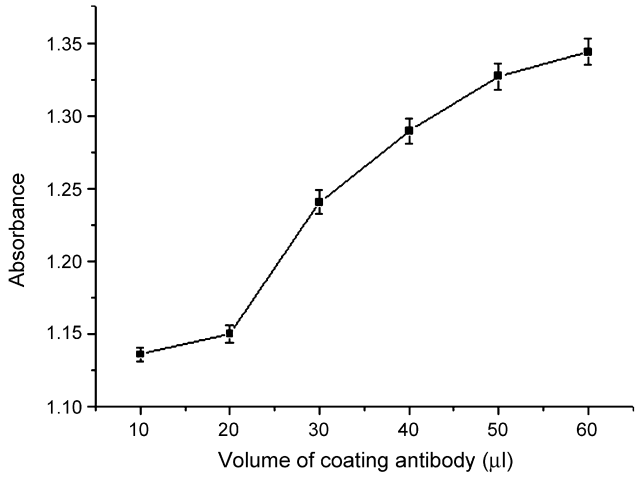


Fig. 1. Effect of the coating amount of goat anti-human IgG antibody (1 mg/ml) per 1 ml colloidal gold on the UV–vis absorption of antibody-colloidal gold conjugate.

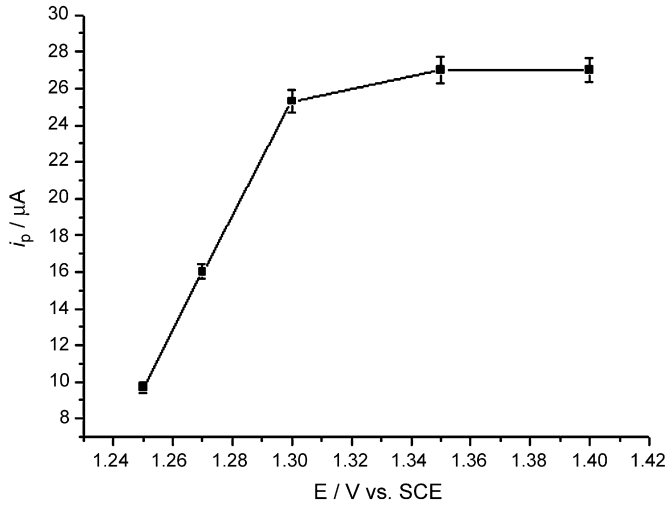


Fig. 2. Effect of the oxidation potential on the reduction wave peak current. Oxidation time, 100 s; IgG concentration, 10 μg/ml.

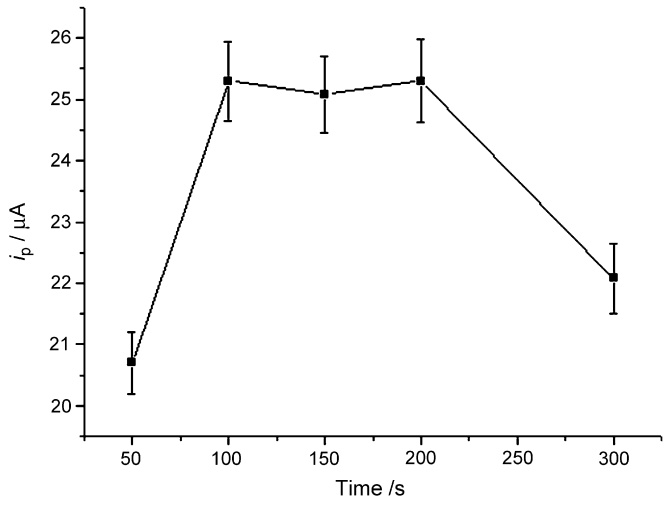


Fig. 3. Effect of the oxidation time on the reduction wave peak current. Oxidation potential: 1.30 V vs. SCE; IgG concentration: 10 μg/ml.

electrochemical cell containing 10 ml of 0.1 M HCl solution. Different oxidation potentials were applied to the immunosensor for 100 s. Then a negative scan from this potential to 0 V with a scan rate of 100 mV/s was applied and the voltammograms were recorded. As shown in Fig. 2, the peak current i_p increases with the increase of the oxidation potential. When the oxidation potential is over 1.30 V, i_p tends to be stable. As a result, an oxidation potential of 1.30 V was selected in the subsequent work to obtain high sensitivity.

3.1.3. Selection of the oxidation time

The oxidation time would also affect the reduction wave peak current heavily. One can presume that insufficient oxidation time would cause incomplete oxidation of the colloidal gold. A redundant oxidation time would result in the diffusing of the $AuCl_4^-$ ions formed out to solution phase. The peak current would decrease under these two extreme conditions. So the appropriate oxidation time should be selected. As shown in Fig. 3, in the range from 100 to 200 s, the reduction wave peak current reaches the highest value, indicating that the colloidal gold was oxidized completely and little $AuCl_4^-$ ions was diffused to the solution phase. Thus, an oxidation time of 150 s was selected.

3.1.4. Selection of the antibody concentration in the adsorption solution

The concentration of the goat anti-human IgG antibody in the coating solution influences the amount of antibody adsorption on the surface of carbon paste electrode. So the concentration of antibody was also investigated in this paper. In order to study the effect of antibody concentration on the resulting immunosensor responses, electrochemically pretreated electrodes were coated with different concentration of goat anti-human IgG antibody for 1 h, followed by blocking with BSA, binding with 10 μg/ml human IgG and antibody-colloidal gold conjugate. The analytical signals were obtained as depicted in Scheme 1. As shown in Fig. 4, the peak current i_p increases with the increase of the antibody concentration. When the concentration is over 0.15 mg/ml, i_p tends to be stable. Therefore, 0.25 mg/ml of goat anti-human IgG antibody was selected for coating the carbon paste electrode.

3.2. The effect of the non-specific adsorption

The effect of the non-specific adsorption was investigated by incubating the immunosensor in 500 ng/ml of human IgG and 1% BSA solution, followed by incubating in antibody-colloidal gold conjugate solution. As mentioned above, the immunosensor was placed in an electrochemical cell and the analytical signal was recorded. As shown in Fig. 5, the binding of human IgG on the immunosensor resulted in more antibody-colloidal gold conjugate bound to the electrode surface, leading to a high reduction wave peak current (curve a). While the immunosensor was incubated with 1% BSA, little colloidal gold was captured to the electrode surface, only a small peak current was observed (curve b), showing the non-specific adsorption was rather low. Without

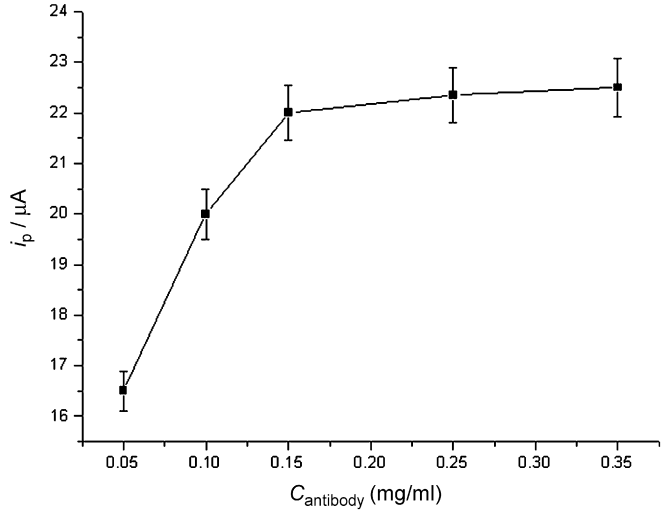


Fig. 4. Effect of the goat anti-human IgG antibody concentration in the adsorption solution on the reduction wave peak current. Oxidation potential: 1.30 V vs. SCE; oxidation time: 150 s; IgG concentration: 10 μg/ml.

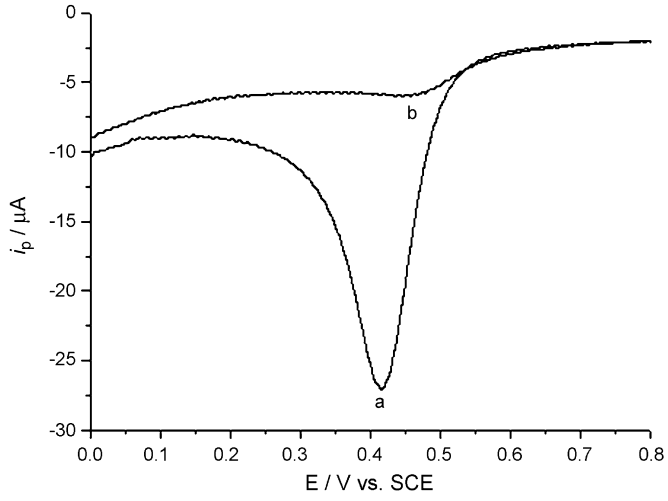


Fig. 5. Voltammograms of the immunosensor response to 500 ng/ml human IgG (a) and 1% BSA (b), respectively.

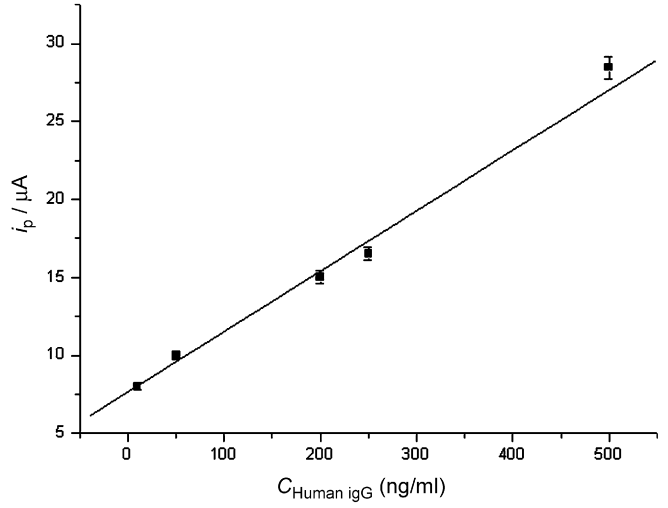


Fig. 6. The calibration curve for the detection of human IgG, indicating that the reduction wave peak current plotted vs. the concentration of human IgG.

much non-specific adsorption, this immunosensor seems to have good selectivity.

3.3. Detection of human IgG

After the immunosensor was incubated with 50 μl of different concentration human IgG and followed by the incubation with antibody-colloidal gold conjugate, the analytical signals were recorded and a linear relationship between the peak current and human IgG concentration was obtained in the dynamic range of 10–500 ng/ml, the linear regression equation was i_p (μA) = 7.322 + 0.0409$C_{human\ IgG}$ with a correlation coefficient of 0.9955 as shown in Fig. 6. The detection limit was 4.0 ng/ml calculated by the 3σ-rule.

4. Conclusion

Colloidal gold label possesses the advantage over radioisotopic or enzyme labels to be stable, and the gold sol labeling procedure is very simple and does not affect generally the biochemical activity of the labeled compound. The electrochemical oxidation of colloidal gold and the oxidation product adsorption on the electrode surface enable the electrochemical detection of the amount of colloidal gold on the electrode surface directly. For each sol particle contains thousands of atoms, this immunosensor shows relative high sensitivity. For the binding force between anti-human IgG antibody and human IgG is relative weak, theoretically the sensitivity of this method for the determination of human IgG should be lower than the detection of streptavidin or biotin. However, this work shows rather high sensitivity as compared with Gonzalez-Garcia's work (the detection limit is 160 and 40 ng/ml for streptavidin and AlbB, respectively) [14]. Probably this was associated with the use of different bonding reagent or the electrochemical pretreatment procedure. It was expected that this new approach can be extended to other immunoassays, DNA hybridization detections and environmental or clinical diagnosis.

Acknowledgment

The work was financially supported by the National Natural Science Foundation of China (Grants No. 20675028, 20435010, 20375012, 20205005).

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