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Femtomolar immunoassay based on coupling gold nanoparticle enlargement with square wave stripping voltammetry

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Abstract

The enhancement in sensitivity for an electrochemical immunoassay by the autocatalytic deposition of Au^{3+} onto gold nanoparticles has been studied. By coupling the autocatalytic deposition with square-wave stripping voltammetry, enlarged gold nanoparticles labeled on goat anti-rabbit immunoglobulin G (GaRIgG-Au) and, thus, the rabbit immunoglobulin G (RIgG) analyte could be determined quantitatively. A variety of variables, such as concentration of $AuCl_4^-$, the reducing agent used, the duration of autocatalytic deposition, and parameters for the stripping analysis were optimized. From a calibration graph over a broad dynamic range of concentrations (1–500 pg mL^{-1}; $R^2 = 0.9975$) a very low detection limit, 0.25 pg mL^{-1} (1.6 fM), which is three orders of magnitude lower than that obtained by a conventional immunoassay using the same gold nanoparticle labels was obtained; this finding confirms applicability and effectiveness of our method of enhancing the sensitivity of gold nanoparticle label-based sandwich immunoassays. The reliability of this method was confirmed by the rather low values of RSD (2.82%, $n = 11$; 2.44%, $n = 9$) obtained for assays of a blank solution and for 0.02 ng mL^{-1} RIgG solution, respectively.

Keywords: Gold nanoparticle labels; Autocatalytic deposition; SWSV; Sandwich immunoassay

1. Introduction

There is great interest in developing new, simple, sensitive, and specific immunoassays for the quantitative determination of analytes of clinical and biological importance. Besides the use of radioactive labels [1], non-radioactive labels for immunoassays have also been developed, including enzymes [2–6], fluorescent molecules [7,8], bio- and chemoluminogenic reagents [9–11], and colloidal metal particles [12–27]. When colloidal metal particles are used as labels for immunoassays and for DNA detection, many analytical methods are available for their quantitative analysis, such as absorption spectrometry [14–16], photothermal deflection spectroscopy [17], surface plasmon resonance spectroscopy [18], Raman spectroscopy [19,20], and electrochemical techniques [23–27]. One of the best detection methods for metal particle label-based immunoassays and DNA detection is electrochemical detection because it offers advantages of low sample volume, excellent sensitivity, and inexpensive instrumentation.

Gold nanoparticles are popular labels for immunoassays and DNA sensing. The deposition of silver or gold onto gold nanoparticle labels has been proposed to enhance the sensitivity of Raman [16,19] and electrochemical [25,27] analyses. Although the sensitivity and detection limits (in tenth of ng mL^{-1}) have been improved, they are not comparable with the powerful enhancement effect of autocatalytic deposition-combined stripping analysis. It is known that the enhancement of sensitivity for an immunoassay or DNA sequence analysis upon depositing silver and gold onto gold nanoparticles is limited by interference of the background current that arises after prolonged deposition of these ions. The reliability of stripping analysis-based detection is affected by the deposition of excess silver, and so the deposition period is limited to a maximum of 11 min [27]. For the deposition of gold on gold nanoparticles, not only the stripping currents but

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also the background currents are increased, and so deposition times shorter than 10 min are used [25].

To explore the effect of autocatalytic deposition on the sensitivity enhancement of an electrochemical immunoassay, we have performed a sandwich immunoassay using gold nanoparticles conjugated to goat anti-rabbit immunoglobulin G (GaRIgG-Au) as an immunoreagent for RIgG. We have studied the experimental conditions related to the autocatalytic deposition to increase the mass of gold nanoparticles labeled on the immunoreagent and have used square wave stripping voltammetry (SWSV) for the analysis of the enlarged gold nanoparticles.

2. Experimental

2.1. Chemicals and reagents

Rabbit immunoglobulin G (RIgG, I-5006), 5 nm gold-conjugated goat anti-rabbit immunoglobulin G, (GaRIgG-Au, G-7277), bovine serum albumin (BSA, 96–99%), sodium azide, formaldehyde (37%), Tween 20, Na_2EDTA, and NaH_2PO_4 were purchased from Sigma Chemical Co. (St. Louis, MO). Bromine, hydrobromic acid (48%), hydrochloric acid (37%), nitric acid (65%), sodium sulfide, NaCl, KOH, NaOH, and K_2HPO_4 were obtained from Merck (Germany). A gold bullion was acquired from Kinebar^{TM} (99.99%, Switzerland). All other chemicals and solvents were of analytical grade and were used as received. All of the solutions were prepared using Milli-Q 18 MΩ water (Millipore purification system).

2.2. Solution preparation and equipment

Standard solutions of RIgG were prepared by the dilution of an RIgG stock solution (10 mg mL^{-1}) using a coating buffer (a solution of PBS containing 0.1% sodium azide). Solutions of GaRIgG-Au were prepared using PBS-T buffer (a solution of PBS containing 0.05% Tween 20). The phosphate-buffered saline (PBS) of pH 7.4 that we used contained 10 mM NaH_2PO_4 and 150 mM NaCl. A 5% BSA solution was used as the blocking agent. To prepare the Au^{3+} solution, a piece of precisely weighed gold bullion was dissolved in aqua regia with stirring and heating. The dissolved solution was diluted with de-ionized water to an appropriate concentration and its pH was adjusted to 7.0 using a solution of KOH. Na_2SO_3 and EDTA were then added to stabilize the Au^{3+} in solution.

The square wave stripping voltammetric analysis (SWSV) was conducted using a CHI 750A electrochemical analyzer (CH Instruments Inc., USA) equipped with an S-7805 stirring machine (Thermolyne Corp., USA). The three-electrode system used for running the SWSV consisted of a glassy carbon working electrode, an Ag/AgCl (3.0 M $NaCl_2$ reference electrode, and a platinum counter electrode. The working electrode was constructed by sealing a small piece of glassy carbon (1.0 mm diameter; 3.0 mm length) into a piece of glass tubing. The polystyrene 96-well microtiter plates (MaxiSorp and PolySorp) were purchased from Nunc (Roskilde, Denmark).

2.3. Electrochemical sandwich immunoassay

The procedure used to perform the sandwich immunoassay using microtiter plates was similar to that reported in the literature [6]. To coat the plate, GaRIgG solution (50 ng mL^{-1}, 400 μL) was added to each well and the plates were then incubated at 4 °C overnight. The coated microtiter wells were washed with PBS-T buffer solutions (3 × 400 μL/well) and the free active sites on the coating microtiter wells were blocked by adding blocking reagent (400 μL/well) to the microtiter wells. The microtiter wells were washed again to remove the un-reacted blocking agent. Standard RIgG solutions (200 μL) were added to each well separately and then the plates were incubated at room temperature for 2 h. After incubation, the microtiter wells were washed again with the same PBS-T buffer solutions and then solutions of immunoreagent (400 μL each of GaRIgG-Au) were added to the microtiter wells. The immunoreaction was then left to proceed at room temperature for 2 h. Un-reacted GaRIgG-Au in the microtiter wells was then removed by washing the microtiter wells with PBS-T buffer solutions (3 × 400 μL/well). To enlarge the gold nanoparticles, Au^{3+} (5.90×10^{-3} M, 200 μL) and formaldehyde (37%, 200 μL) solutions were added to each microtiter well, which was then stirred gently with the micropipette. The electrolytic solutions were removed from the microtiter wells after autocatalytic deposition of gold for 90 min. The microtiter wells were washed with PBS-T buffer (3 × 400 μL/well). A 1.0 M HBr/0.1 mM Br_2 solutions (400 μL) was added to each microtiter well to dissolve the enlarged gold nanoparticles. A portion of each dissolved solution (80 μL) was then transferred to an electrolytic cell for SWSV analysis.

To maintain the activity of the GC working electrode during SWSV, the electrode surface was conditioned periodically by applying a potential of +1.200 V for 3 min. SWSV was performed by applying a deposition potential of −0.8 V for 1 min while stirring gently. The deposited gold was then stripped by sweeping the potential from 0.000 to 0.900 V with a potential step of 4 mV. The amplitude and frequency of the square wave used were 25 mV and 15 Hz, respectively. It took about 15 s to perform the whole stripping process. The square wave stripping voltammograms obtained were processed using Sigma Plot software.

3. Results and discussion

3.1. Sensitivity enhancement for Au^{3+} analysis

To effect autocatalytic deposition, formaldehyde was used as a reducing agent to promote the reduction of Au^{3+}. To op-