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Silver deposition directed by self-assembled gold nanorods for amplified electrochemical immunoassay

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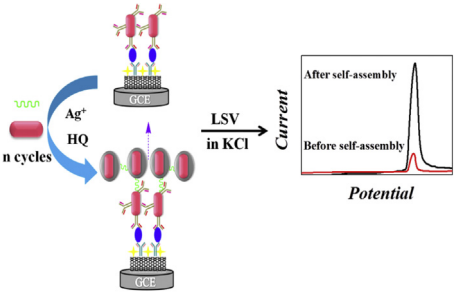
HIGHLIGHTS

- An ultrasensitive nonenzymatic electrochemical immunosensor for HIgG detection was developed.
- AuNRs were used to catalyze the deposition of silver.
- Detection signal was greatly amplified by self-assembly of AuNRs and the subsequent silver enhancement.
- This immunosensor exhibited a extremely low detection limit.

GRAPHICAL ABSTRACT



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ABSTRACT

A novel electrochemical immunoassay was developed based on the signal amplification strategy of silver deposition directed by gold nanorods (AuNRs), which was in-situ assembled on the sandwich immunocomplex. The superstructure formed by the self-assembly of AuNRs provided abundant active sites for the nucleation of silver nanoparticles. In this pathway, the stripping current of silver was greatly enhanced. Using human immunoglobulin G (HIgG) as a model analyte, the ultrasensitive immunoassay showed a wide linear range of six orders of magnitude from 0.1 fg mL^{-1} to 100 pg mL^{-1}, with the low detection limit down to 0.08 fg mL^{-1}. The practicality of this electrochemical immunoassay for detection of HIgG in serum was validated with the average recovery of 93.9%. In addition, this enzyme-free immunoassay also has the advantages of acceptable reproducibility and specificity, and thus this immunosensing protocol can be extended to the detection of other low-abundant protein biomarkers.

1. Introduction

In recent years, electrochemical immunoassays for the detection of disease biomarkers have gained increasing attention due to their intrinsic superiority, such as fast speed and low cost, which can favorably meet the clinical requirements [1–3]. What's more, electrochemical immunoassay can offer high sensitivity and low detection limit owing to the convenient incorporation of various signal amplification strategies into the assay protocol [4–9]. Of particular interest is the gold nanoparticles (AuNPs)-catalyzed silver deposition because of its inherent signal amplification nature induced by the deposited silver [4,10].

Among various shapes of AuNPs, gold nanorods (AuNRs) attracted substantial attention in recent several years [10–12]. In addition to the unique optical properties [13,14], AuNRs possess

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general properties similar to AuNPs such as excellent conductivity and biological compatibility, and have several advantages over the spherical AuNPs, including fast electron transfer rate and high surface area [15,16], which inspired researchers to explore the application of AuNRs in electrochemical biosensors [17–19]. Zang et al. [17] established an electrochemical immunosensor for sensitive detection of ofloxacin based on multi-enzyme-antibody functionalized AuNRs. The study of Li's group [18] exhibited that the sensitivity of the proposed immunosensor relied on the amount of label and antibody conjugated on the AuNRs. Du et al. [19] demonstrated that AuNRs could link enzyme and detection antibody at high ratio. These works indicated that AuNRs were excellent nanocarriers for the biomolecular recognition events. Recently, Ju's group [5] developed an immunosensing amplification strategy with ultralow detection limit for α-fetoprotein, by introducing a host–guest binding reaction into the assembly process of AuNRs superstructure. Thus, the AuNRs based superstructure provided a new avenue for signal amplification in the design of electrochemical immunosensors. Compared with the relatively positive electrochemical oxidization potential of gold, metal silver can be oxidized at a lower potential with a sharp stripping peak [20]. Thus, AuNPs-mediated silver enhancement after specific formation of sandwich immunocomplex has been proposed for sensitive electrochemical immunoassay [3,4].

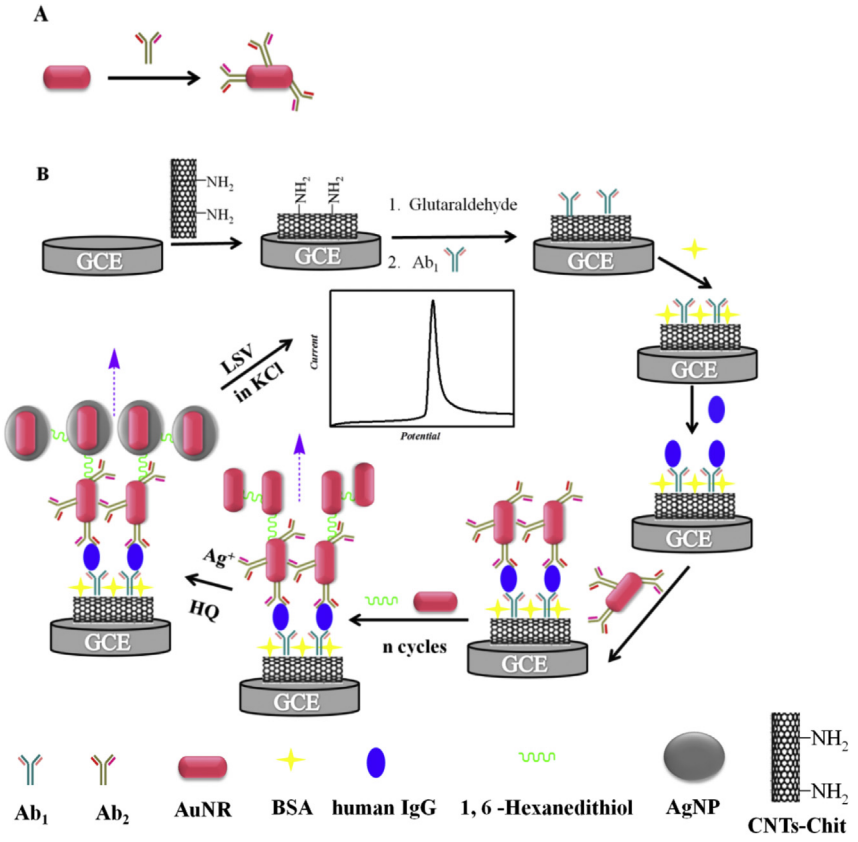
Herein, AuNRs-directed silver deposition strategy was applied to design an ultrasensitive electrochemical immunosensor. As shown in Scheme 1, the carbon nanotubes-chitosan composite (CNTs-Chit), owing to excellent conductivity and favorable biocompatibility, was utilized to immobilize the capture antibodies and catalyze the electrochemical oxidation of the deposited silver [5,21,22]. After the formation of sandwich-type immunocomplex, AuNRs was introduced onto the surface of the immunosensor. Then, the multi-layer AuNRs was assembled via the strong covalent Au–S linkage. After that, the superstructure-directed silver deposition was performed. Target proteins were quantitatively analyzed using the amplified signal of silver. Using human immunoglobulin G (HIgG) as a model analyte, the proposed method showed a detection limit down to ag mL^{-1} level.

2. Experimental

2.1. Materials and reagents

HIgG, mouse anti-human IgG antibody (anti-HIgG) and bovine serum albumin (BSA) were obtained from Beijing Biosynthesis Biotechnology Co. Ltd. (Beijng, China). Chitosan (Chit, MW 5–6 × 10^5, >90% deacetylation) was purchased from Shanghai Yuanju Biotechnology Co. Ltd. (Shanghai, China) and was used as fixative. Carboxylated CNTs (purity >95wt%, Outer diameter 30–50 nm, and length 20 μm) were purchased from Chengdu Organic Chemicals Co. Ltd. (Chengdu, China). Glutaraldehyde (GA), L-ascorbic acid (AA) and hydroquinone (HQ) were purchased from Kemiou Chemical Reagent Co. Ltd. (Tianjin, China). Cetyltrimethylammonium bromide (CTAB), Chloroauric acid, trisodium citrate, and silver nitrate were obtained from Shanghai Reagent Company (Shanghai, China). Sodium borohydride was obtained from Sinopharm Chemical Reagent Co. Ltd. (China). Tween-20 was obtained from MP Biomedicals. All other reagents were of analytical grade and used as received.

Phosphate buffered saline (PBS, 0.01 M, pH 7.4) was prepared by mixing $Na_2HPO_4 \cdot 12H_2O$ and KH_2PO_4. 0.01 M PBS containing 1.0% (w/v) BSA was used as blocking solution. Washing solution (PBST) was prepared by dissolving 0.05% (V/V) Tween-20 in PBS [7]. Deionized and distilled water was used throughout the study.



Scheme 1. Schematic representation of the preparation of (A) AuNRs-Ab_2, (B) the electrochemical immunosensor and sandwich immunoassay procedure.