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Ultrasensitive colorimetric immunoassay for hCG detection based on dual catalysis of Au@Pt core–shell nanoparticle functionalized by horseradish peroxidase

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ABSTRACT

In this paper, an ultrasensitive colorimetric biosensor for human chorionic gonadotrophin (hCG) detection was designed from bottom-up method based on the dual catalysis of the horseradish peroxidase (HRP) and Au@Pt nanoparticles (NPs) relative to H_2O_2-TEM system. HRP and monoclonal mouse anti-hCG antibody (β-submit, mAb_1) were co-immobilized onto the Au@Pt NP surface to improve catalytic efficiency and specificity, which formed a dual functionalized Au@Pt-HRP probe with the mean size of 42.8 nm (D_{50}). The colorimetric immunoassay was developed for the hCG detection, and the Au@Pt-HRP probe featured a higher sensitivity in the concentration range of 0.4–12.8 IU L^{-1} with a low limit of detection (LOD) of 0.1 IU L^{-1} compared with the LODs of 0.8 IU L^{-1} for BA-ELISA and of 2.0 IU L^{-1} for Au@Pt, which indicated that the Au@Pt-HRP probe possessed higher catalytic efficiency with 2.8-fold increase over Au@Pt and 33.8-fold increase over HRP. Also, the Au@Pt-HRP probe exhibited good precision and reproducibility, high specificity and acceptable accuracy with CV being less than 15%. The dual functionalized Au@Pt-HRP probe as a type of signal amplified method was firstly applied in the colorimetric immunoassay for the hCG detection.

1. Introduction

Accurate detection of biomarkers is important in the early diagnosis [1]. In the last several decades, an abundance of assay methods have been reported for the detection of proteins, including radioimmunoassay [2], enzyme-linked immunosorbent assay (ELISA) [3], fluorescence immunoassay [4–5], chemiluminescence immunoassay [6], and electrochemical immunoassay [7]. ELISA, possessing the low limit of detection (LOD) ranged from 0.1 to 1000 ng mL^{-1}, is a general method as the gold standard among these methods [8–11]. Nonetheless, the biomarker content in the sample from patient at an early stage of diseases is sometimes lower than the LOD of ELISA. Up to date, various signal amplification approaches have been developed to enhance the sensitivity and to increase the LOD of ELISA. Signal amplification based on nanomaterials, such as platinum nanopartilces [7], gold nanoparticles [12], magnetic beads [13], PAMAM [14], carbon nanotubes [15] and mesoporous silica [16], has recently attracted considerable attention. The nanomaterials can not only provide amplified recognition events by high loading of signal tags, but also produce catalytic activity [17]. Among various nanomaterials, platinum nanoparticles and its derivatives are frequently applied the bioassays due to their advantage such as high catalytic activity, high stability, and good biological compatibility [18–19]. For example, Gao et al. reports a new colorimetric immunoassay based on platinum nanoparticles as a substitution of horseradish peroxidase (HRP) with the LOD of 2.5 ng mL^{-1} toward rabbit immunoglobulin G (IgG) [20], which is higher than 1.0 ng mL^{-1} for HRP. Nowadays, some reports have presented that platinum-based bimetallic nanocatalysts, such as Au@Pt nanoparticles, are more active for a series of catalytic reactions compared with single platinum particles due to the change in the electronic structure and surface atomic arrangement [21]. Au@Pt nanoparticles even behaves a higher catalytic activity than HRP in oxidation reaction of 3, 3′,5,5′-tetramethylbenzidine (TMB) by H_2O_2. Therefore, Au@Pt nanoparticle is a good platform for the signal amplification in ELISA.

Although Au@Pt nanoparticle features high catalytic activity as nanocatalysts, the high loading activity which can load a large number of signaling molecules or molecules that can generate signals indirectly

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(HRP for example) was always neglected. Therefore, design concept of dual catalysis based on Au@Pt NPs and HRP for ultrasensitive detection of human chorionic gonadotrophin (hCG) is interesting and meaningful in the signal amplification of ELISA.

2. Experimental

2.1. Material and Reagent

Monoclonal mouse anti-hCG antibody α-submit (mAb_2) and β-subunit (mAb_1, Artron BioResearch Inc., Burnaby, British), hCG standards (Beijing Jiashiyuhe research institute of chemical technology Co., Ltd., Beijing, China), sodium citrate tribasic dihydrate (Shanghai Titanchem. Co., Ltd., Shanghai, China), L-ascorbic acid (Aladdin industrial corporation, Shanghai, China), chloroauric acid tetrahydrate ($HAuCl_4 \cdot 4H_2O$, 48–50% Au basis) and potassium tetrachloroplatinate (Shanghai Macklin Biochemical Co., Ltd.), Horseradish peroxidase (HRP, Sigma-Aldrich, St. Louis, MO), 3,3′,5,5′-tetramethylbenzidine (TMB, Aladdin industrial corporation, Shanghai, China), and polystyrene microplates (Costar, Corning Inc., New York, USA, 3590) were used without pretreatment. The other reagents used in this work were analytical grade.

Coating buffer: 50 mM sodium carbonate/bicarbonate, pH 9.6; Blocking buffer: 10 mM PBS including 1 wt% BSA, pH 7.4; Washing buffer: 10 mM PBS including 0.5 wt% Tween 20, pH 7.4.

2.2. Preparation of Gold@Platinum Nanoparticles (Au@Pt NPs)

Au@Pt NPs were prepared according to the report previously with a minor modification [22]. In briefly, 1 mL aqueous sodium citrate tribasic (1 wt%) was added to the boiling double distilled solution (99 mL) including $HAuCl_4$ (1 mL, 1 wt%). The solution was kept boiling for 30 min to provide the gold nanoparticles (Au NPs). The volume of Au NP suspension was finally adjusted to 100 mL. The Au NP concentration of the suspension was accordingly calculated to approximately be 2.4×10^{-10} mol L^{-1}. Then, 33 mL aqueous K_2PtCl_4 solution (1.0 mM) was added to the as-prepared suspension at 80 °C and stirred. Afterwards, 16 mL aqueous L-ascorbic acid solution (10 mM) was added to the mixture to reduce K_2PtCl_4 for another 30 min with the color change from red to dark. The concentration of Au@Pt NP suspension was accordingly calculated approximately to be 1.6×10^{-10} mol L^{-1}. (The detailed calculation process was listed in the Supplementary Information.).

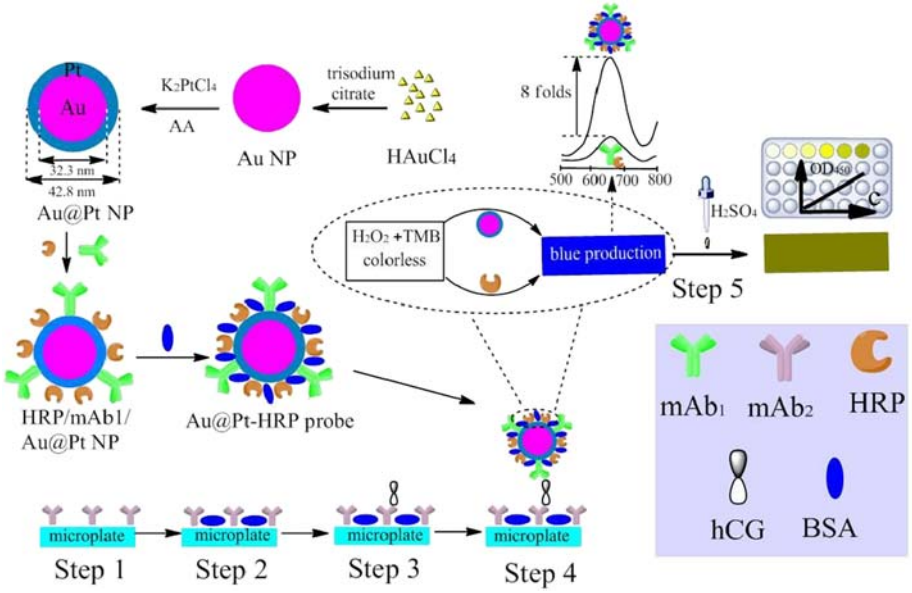
2.3. Preparation of Au@Pt NP Probe (Au@Pt-HRP Probe)

As-synthesized Au@Pt NP suspension (1.5 mL) was adjusted to pH 8.5–9.0 with K_2CO_3 solution (0.2 M). Then, 7.2 μL mAb_1 (0.5 mg mL^{-1}) and HRP (4.8 μL, 5 mg mL^{-1}) were injected to the above Au@Pt NP suspension, and vibrated for 2 h at room temperature. Afterwards, 260 μL BSA (10 wt%) was added into the mixture to keep the BSA content to be 1.7 wt%, and incubated for another 16 h at 4 °C. The Au@Pt-HRP probe was collected and rinsed with Tris-HCl buffer (50 mM) containing 1.0 wt% BSA (pH 8.8) (1 mL per time) for three times in consecutive washing/centrifugation cycles, and dispersed into 0.5 mL Tris-HCl buffer (50 mM) containing 1.0 wt% BSA (pH 7.6). The Au@Pt-HRP probe was finally stored at 4 °C for the following immunoassay.

2.4. Detection of Target hCG

The protocol for the detection of hCG was listed in Scheme 1. The solid-phase antibody was prepared by injecting mAb_2 in the coating buffer (100 μL, 1.5 μg mL^{-1}) into a high-binding 96-well microplate and incubating for 12 h at 4 °C. The microplate was then rinsed with washing buffer (200 μL per well) and blocked with blocking buffer (200 μL per well). The microplate was then rinsed with washing buffer (200 μL per well) to provide the solid-phase antibody for the following detection.

The Au@Pt-HRP probe was then applied for the detection of hCG as following: Firstly, 100 μL hCG standard sample was injected to the solid-phase antibody and maintained at 37 °C for 60 min. Secondly, after washing with washing buffer for three times (200 μL per well per time), Au@Pt-HRP probe (100 μL) was added into each well, and reacted at 37 °C for another 60 min. Thirdly, after washing for three times (200 μL per well per time), TMB substrate (100 μL) was added into each well, and incubated for 30 min at 37 °C. Finally, the catalytic reaction was stopped by using H_2SO_4 (50 μL, 2 M). The absorbance was recorded at 450 nm using a microplate reader (SpectraMax190, Molecular Devices LLC., Sunnyvale, California.). All the results were repeated for three times.



Scheme 1. The colorimetric immunoassay protocol of Au@Pt-HRP probe.