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Peptide aptamer-based biosensor for the detection of human chorionic gonadotropin by converting silver nanoparticles-based colorimetric assay into sensitive electrochemical analysis

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ABSTRACT

We presented an antibody-free electrochemical technique for the determination of human chorionic gonadotropin (hCG) using silver nanoparticles (AgNPs) as the redox reporters and a hCG-specific binding peptide as the receptor. Peptide-induced AgNPs assembly was achieved on the electrode surface that was modified with the same sequence of peptide (recognition element) used in the AgNPs aggregation. As a result, a well-defined linear-sweep voltammetry (LSV) peak was observed. The attachment of hCG on the electrode surface made the peptide probe lose its ability to trigger the *in situ* formation of the AgNPs-based network architecture on the electrode surface, thus leading to a much attenuated LSV current. The current decreased with the increase of hCG concentration ranging from 1 mIU/mL to 0.2 IU/mL. Consequently, a detection limit of 0.4 mIU/mL was achieved. To demonstrate the amenability of our method, the contents of hCG in human serum/urine samples were determined. The results were consistent with those obtained by the enzyme linked immunosorbent assay (ELISA). Based on the well-defined and amplified electrochemical signal of the AgNPs-based network architecture, our work would also be valuable for the design of novel electrochemical sensors by marrying specific receptors.

1. Introduction

Human chorionic gonadotropin (hCG) is a hormone produced by implanted embryo. It has also been suggested that elevated level of hCG was found in some cancerous tumors (*e.g.* prostate tumor, testicular tumors, trophoblastic tumor and gestational choriocarcinoma) [1,2]. Thus, hCG has been considered to be a molecular biomarker for the diagnosis of pregnancy and some cancers. The current used lateral-flow immunoassay (hCG diagnostic kit) is simple to use and shows a qualitative result with a detection limit of ~20 mIU/mL in urine [3]. However, this technique is difficult to quantify low levels of hCG in the body fluids of cancer patients. For this view, a number of immunoassays have been presented for quantitative analysis of hCG in the past few years, such as fluorescent immunoassays [4], radioimmunoassays [5], enzymelinked immunosorbent assay (ELISA) [6], electrochemiluminescence [7], surface plasmon resonance (SPR) [8] and electrochemical immunosensors [9–11]. These techniques are sensitive and reliable for hCG determination. However, they are either expensive or time-consuming and require the utilization of less stable antibody that is vulnerable to environmental factor. Therefore, for the detection of hCG in body fluids, it is still desirable to develop antibody-free detection techniques with the merits of cheapness, convenient manipulation and high sensitivity.

Peptide aptamer is a promising candidate to replace antibody as a molecular receptor for biosensing, because it is more stable and resistant to harsh environments and readily synthesized with the desired sequence for binding the specific target [12–15]. Recently, a few engineered peptide aptamers have been found and used as the recognition elements for binding to various proteins [3,16–24]. For example, Yang et al. found that the peptide with a sequence of PPLRINRHILTR shows a strong interaction with hCG (K_D = 0.9 nM), and thus reported an antibody-free liquid crystal (LC) assay for hCG detection [3]. The findings give researchers a hint that the hCG-binding peptide could be used as a receptor for the development of antibody-free hCG biosensors. Over the past decade, Au/Ag nanoparticles-based liquid-phase colorimetric assays have received considerable attention in chemical and

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biomedical fields due to their simple detection principles and easy manipulation procedures for signal readout [25–27]. Lin and coworkers have thus developed two colorimetric biosensors based on the specific aptamer-hCG interaction and the good catalytic and optoelectronic properties of gold naoparticles (AuNPs) [21,28]. Specifically, the hCG-specific peptide aptamer could absorbed onto the surface of AuNPs to induce the aggregation and color change of AuNPs suspension, thus inhibiting the catalytic ability of AuNPs to promote the conversion of a yellow-colored 4-nitrophenol to a colorless product 4-aminophenol. However, when hCG was present in the solution, the peptide aptamer specifically bound to hCG and lose its ability to induce the aggregation of AuNPs. Thus, the monodisperse AuNPs remained red and restored their catalytic activity.

The unmodified Au/Ag nanoparticles-based colorimetric sensing technique is simple and does not require modification of any analyte-binding molecules onto the surface of nanoparticles. However, the unmodified method exhibits poor anti-interference ability for the selective detection of proteins in biological fluids because some matrix components in real samples may absorb onto the surface of bare Au/Ag nanoparticles [21,29,30]. Moreover, most of the colorimetric assays show low sensitivity (usually with the detection limits at nanomolar scale or over). Thus, the colorimetric examples were expected to re-create existing platforms with improving sensitivity. Very recently, Dai's group proposed a novel concept for converting a liquid-phase colorimetric assay into an enhanced surface tethered electrochemical analysis [31], which is based on the Hg^{2+}-thymine-induced formation of a network architecture of silver nanoparticles (AgNPs) on electrode surface. This strategy takes the advantages of simple principle and easy operation of colorimetric assays as well as the virtues of excellent sensitivity, fast response and compatibility with miniaturization of electrochemical sensors. We have demonstrated the competitive electrochemical assay of amyloid-β oligomers (AβOs) by employing AgNPs as the redox reporters and an AβOs-specific adamantine (Ad)-labeled peptide as the receptor [30]. In that assay, the network architecture of peptide-AgNPs were produced in solution and then introduced to the β-cyclodextrin (β-CD)-modified electrode surface through the host–guest interaction. The specific peptide-AβOs interaction made the peptide losing its capability to induce the formation of AgNPs-based network architecture in solution. However, this method requires the modification of both electrode and peptide probe. More importantly, the method could not improve the poor anti-interference ability of bare AgNPs-based assays of real samples.

In the present work, we found that the hCG-binding peptide can induce the aggregation and color change of AgNPs, whereas hCG prevented the peptide-triggered AgNPs aggregation. Since gold electrode exhibits a superficial microenvironment similar to that of Au/Ag nanoparticles, we suggested that the aggregation of AgNPs in the solution would be facilely initiated on a solid (electrode)-liquid (electrolyte) surface by modifying the electrode with the peptide probe. Considering that AgNPs aggregates show a well-defined and amplified electrochemical signal, we demonstrated that the AgNPs-based colorimetric assay could be developed into an electrochemical platform for the sensitive and selective detection of hCG. The use of dual-functioning peptide (binding to hCG and inducing AgNPs aggregation) obviated the utilization of expensive and less stable antibodies and the modification of any analyte-binding receptors onto the surface of nanoparticles. Moreover, the conversion of colorimetric assay into electrochemical platform not only improved the sensitivity and selectivity but also maintained the simple detection principles and easy manipulation procedures. This work provides a hint for the design of novel AgNPs-based electrochemical sensors by marrying specific receptors.

2. Experimental

2.1. Chemicals and reagents

Human chorionic gonadotropin (hCG), bovine serum albumin (BSA), thrombin, immunoglobin G (IgG), tris(carboxyethyl)phosphine (TCEP) and 6-mercapto-1-hexanol (MCH) were purchased from Sigma-Aldrich (Shanghai, China). Follicle-stimulating hormone (FSH), luteotropic hormone (LH) and thyroid stimulating hormone (TSH) were provided by WuHan AmyJet Scientific Inc. (Wuhan, Hubei, China). Beta-subunit of hCG (β-hCG) was obtained from YuduoBio Co., Ltd. (Shanghai, China). Trisodium citrate was obtained from Sangon Biotech. Co., Ltd. (Shanghai, China). Silver nitrate ($AgNO_3$) was purchased from Aladdin Reagent Company (Shanghai, China). Peptides used in this work were synthesized and purified by China Peptides Co., Ltd. (Shanghai, China). The peptide stock solution at a concentration of 1 mM was prepared with deionized water and diluted to a given concentration with a phosphate-buffered saline solution (PBS buffer, 2 mM, pH 7.2) before use. All other chemicals were of analytical grade and provided by Beijing Chemical Reagent Co. Ltd (Beijing, China). All the electrochemical measurements were performed on a CHI 660E electrochemical workstation (CH Instruments, Shanghai, China) with a platinum wire and an Ag/AgCl electrode as the auxiliary and reference electrodes, respectively.

2.2. Synthesis of AgNPs

AgNPs were prepared through the chemical reduction of Ag^+ ions with $NaBH_4$ as the reducing reagent and trisodium citrate as the stabilizer. Briefly, 1 mL of a 10 mM $AgNO_3$ solution and 1 mL of a 10 mM trisodium citrate solution were added to 36.8 mL of deionized water under vigorous stirring. It was followed by addition of 1.2 mL of freshly prepared 10 mM $NaBH_4$. The solution color gradually changed to yellow, indicating the formation of the AgNPs. After the reaction proceeded for approximately 10 min, the resulting colloid was aged for 2 days at 4 °C. The morphology of AgNPs was observed by a FEI Tecnai G2 T20 transmission electron microscope (TEM). The average sizes of AgNPs were measured on a Nano ZS laser scattering particles size analyzer (Malvern Instruments Ltd., Malvern, Worcester-shire, UK). The concentration of the AgNPs was calculated to be 4.3 nM according to the AgNPs size and the Ag^+ concentration. Before use, the AgNPs were diluted to 2 nM with 2 mM PBS at pH 7.2.

2.3. Colorimetric assays

250 μL of the diluted AgNP dispersion at a concentration of 2 nM was added to 250 μL of 100 nM peptide (PPLRINRHILTR) solution. After incubation for 5 min, color change was observed with the naked eye, and the UV–vis absorption spectra were recorded with a Cary 50 spectrophotometer using a 1 cm quartz spectrophotometer cell. The photograph of AgNPs suspension was taken with a digital camera. To investigate the effect of hCG on peptide-triggered AgNPs aggregation, 200 μL of peptide solution was mixed with 50 μL of hCG solution in a test tube. Then, the mixture was incubated with 250 μL of the diluted AgNPs at room temperature for 5 min. The absorption spectra and the photographs of the mixed solution were recorded with the UV–vis spectrophotometer and the digital camera, respectively.

2.4. Preparation of sensing electrode

Gold electrodes with 3-mm diameter (www.gaossunion.com) were polished with diamond pastes down to 0.3 μm and alumina pastes down to 0.05 μm. The cleaned electrodes were then