Irregularly Shaped Gold Nanoparticles as Secondary Labels for Enhanced Electrochemical Sandwich-type Enzyme Immunoassay for the Determination of Biomarkers

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This letter describes a dual-amplified electrochemical sandwich-type enzyme immunoassay for the cancer antigen 125 (CA125) by using irregularly shaped gold nanoparticle-labeled horseradish peroxidase (HRP)-bound anti-CA125 as secondary antibodies. Compared with conventional sandwich-type immunoassays, the proposed immunoassay exhibits a low detection limit and wide linear range.

An ultrasensitive and simple method for detecting and quantifying biomarkers is essential for early diagnosis of diseases in modern medicine.[1] Despite many advances in this field, it is still a challenge to find new approaches that could improve the simplicity, selectivity, and sensitivity of clinical immunoassays. Some promising approaches are to employ enzymes,[2] nanoparticles,[3] DNA-based polymerase chain reaction (PCR),[4] and liposome-PCR assay[5] as amplified signal reporters. Although all DNA-based immunoassays could display very low detection limits, their practical application is restricted by the complex detection procedures or conjugation chemistries.[6]

Recently, nanoparticles have received wide attention as electrocatalysts for electrochemical reaction.[6] Spherical gold nanoparticles with simple chemical preparation, a narrow size distribution, and efficient coating by thiols or other bioligands have been used as transducers for several biorecognition binding applications, such as bioconjugation with antibodies.[7] However, gold nanoparticles with different shapes possessed various catalytic efficiency.[3] Li et al. found that the specially shaped, irregular gold nanoparticles had higher catalytic efficience on luminol chemiluminescent than that of spherical gold nanoparticles as labels.[3] In addition to nanoparticles, enzymes, such as HRP or alkaline phosphatase (AP), are utilized as labels for biocatalytic amplification. Wilson and Nie reported a series of electrochemical enzyme immunoassays by covalently binding or physically adsorbing analytes on iridium oxide electrodes to recognize AP-labeled antibodies.[2] To date, however, there is little reports focusing on electrochemical studies for the antigen–antibody reaction by simultaneously using gold nanoparticles and HRP as labels.

Here, we report an ultrasensitive and simple electrochemical immunoassay method for the detection of CA125 in human by using a new double-codified nanolabel based on irregularly shaped gold nanoparticle modified HRP-bound anti-CA125 antibodies (*bionanoconjugate*, Figure 1) as secondary antibodies. The detection is based on the catalytic reduction of the carried HRP relative to the H_2O_2 system with the sandwich-type immunoassay format.

For the preparation of a sandwich-type heterogeneous electrochemical immunosensor, an anti-CA125 layer was formed on an indium tin oxide (ITO) electrode (Figure 1). Partially ferrocenyltethered dendrimer (D-Fc)[8] was initially immobilized onto the ITO electrode by covalent bonding between dendrimer amines and carboxylic acids of a phosphonate self-assembled monolayer, and then anti-CA125 antibodies were covalently bound to the unreacted amines of D-Fc by the glutaraldehyde (GA). The specially shaped gold nanoparticles with an mean length of ≈50 nm and a narrow width of 27 nm along its longitudinal axis were prepared according the literature.[3] Briefly, 50 mL of $HAuCl_4$ aqueous solution (0.1 mM), 5 mL of starch (0.17%, w/w), and 1 mL of D-glucose (1.0 mM) were added into a 150-mL round-bottom flask of and kept stirring at 40 °C for 24 h until the mixture turned light violent. The double-codified gold nanoparticles as secondary antibodies were prepared via direct adsorption of HRP-labeled anti-CA125 on the surface of gold nanoparticles.[9] This simple conjugation method is effective in retaining HRP–anti-CA125 activity after conjugation.[10] The double-codified bionanoconjugates and the immunosensing layer sandwich the target protein (CA125).

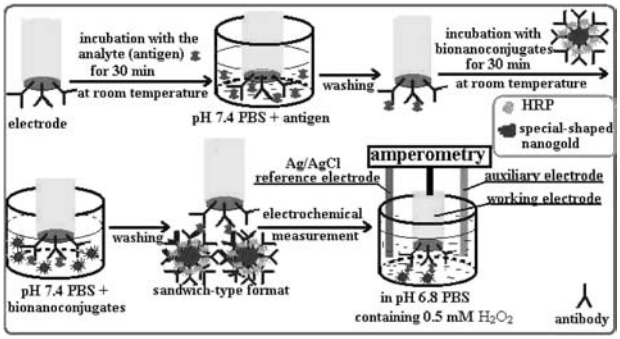

Figure 1. Measurement process of the electrochemical sandwich-type enzyme immunoassay.

First, we investigated the amperometric responses of the sandwich-type immunoassay by using the as-prepared bionanoconjugates as secondary antibodies relative to the H_2O_2–PBS (pH 6.8) after the antigen–antibody reaction. With the addition of 0.5 mM H_2O_2 into the electrochemical cell, the cathodic peak increased dramatically and the anodic peak decreased (Figure 2). The catalytic current is mainly due to the electron transfer between the labeled bionanoconjugate and the electrode, and the electron mediation of ferrocene acts as the bridge to provide an electrical contact or a pathway of electron transfer between the immobilized bionanolabels and the base electrode. The reason for the large current response might be attributed to the amplification of enzymatic bioelectrocatalysis signalling. Meanwhile, the irregular gold nanoparticles could enhance the immobilized amount of the labeled enzyme, which exhibited higher catalytic efficiency.

To accomplish an ultrahigh sensitivity, a high signal-to-

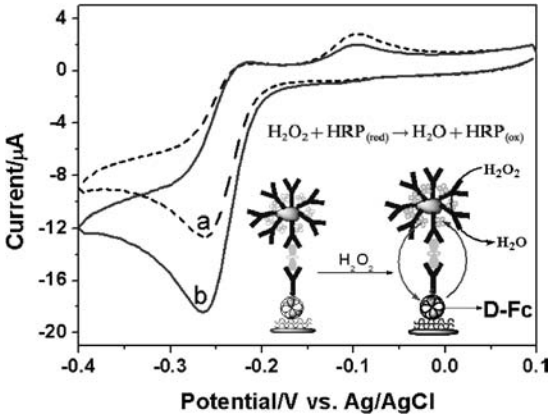

Figure 2. Cyclic voltammograms of the formed sandwich-type immunosensor in the absence (a) and presence (b) of 0.5 mM H_2O_2 into the detection solution of pH 6.8 PBS after reacted with 20 U/mL CA125.

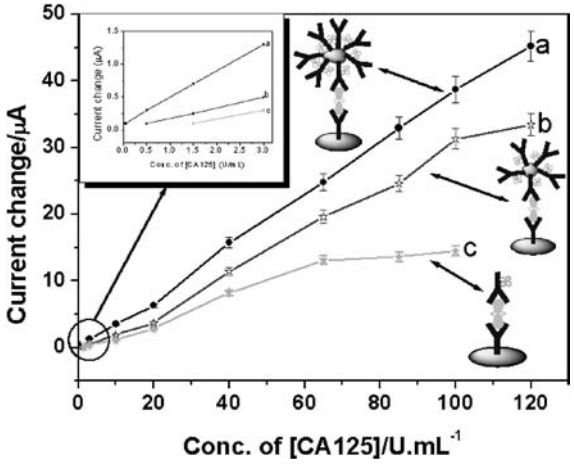

Figure 3. Calibration curves for the electrochemical sandwich-type immunoassay of CA125 recorded using bionanoconjugates (a), spherical nanogold-bound HRP–anti-CA125 (b), and HRP–anti-CA125 as labels.

noise ratio is required. A cyclic voltammetric measurement with a sandwich immunoassay format was employed to detect CA125 with the developed bionanoconjugates as tracer and H_2O_2 as substrate. The current responses increased with the increment of CA125 concentration in the sample solution after the antigen–antibody interaction in H_2O_2–PBS system (pH 6.8). The increase of reduction current was proportional to CA125 concentration in the range of 0.05 to 120 U/mL, and the linear regression equation is i_{pc} (μA) $= -0.1013 + 0.3831 \times C_{[CA125]}$ (U/mL) with a detection of 0.01 U/mL (Figure 3a) at a signal to noise ratio of 3δ (where δ is the standard deviation of a blank solution, $n = 11$) ($R^2 = 0.987$). For comparison, we also used the 41-nm spherical gold nanoparticle-bound HRP–anti-CA125 and HRP–anti-CA125 as secondary antibodies for CA125 detection following the same protocols, and the linear ranges and detection limits are 0.5–100 U/mL with a detection of 0.1 and 1.5–65 U/mL with a detection of 0.5 U/mL CA125, respectively (Figures 3b and 3c). It revealed that the proposed bionanoconjugate labeling method possessed the advantages of higher sensitivity and wider linear range compared with those of directly using HRP and spherical nanogold particles as labels. The reason might be the fact that the irregular gold nanoparticles exhibited larger surface than that of spherical nanoparticles, which could immobilize more HRP–anti-CA125 biomolecules.

For heterogeneous phase immunosensors, nonspecific binding of nontarget proteins to the immunosensing layer could also be a source of noise. To investigate the specificity of the proposed immunosensor, α-1-fetoprotein (AFP), CA 19-9, CEA, and BSA were used in this study. Amperometric responses of the proposed immunoassay in 0.5, 20, 80, and 100 U/mL of CA125 solutions containing interfering substances of different concentrations were assayed, and the RSD values were 3.4–9.7%, 4.3–9.1%, 2.9–8.7%, and 3.2–9.4%, respectively. So the selectivity of the developed immunoassay was acceptable. The stability of the immunoassay system was examined. When the immunosensor was dried and stored at 4 °C, it retained 90.2% of its initial response after a storage period of 16 days. The slow decrease of response seemed to be related to the gradual deactivation of the immobilized antibody incorporated in the composite.

The reproducibility of the immunoassay system was evaluated by intra- and interassay coefficients of variation (CVs). The intra-assay precision of the analytical method was evaluated by analyzing four concentration levels five times per run in 6 h. The CVs of intra-assay with this method were 3.7, 5.3, 6.2, and 5.9% at 0.5, 20, 80, and 100 U/mL of CA125, respectively. Similarly, the interassay CVs on five immunosensors were 4.8, 7.3, 6.8, and 5.7% at 0.5, 20, 80, and 100 U/mL of CA125, respectively. Thus, the precision and reproducibility of the proposed immunoassay was acceptable.

In summary, we described a double-codified electrochemical sandwich-type immunoassay with an ultrahigh sensitivity by using HRP and nanogold amplification. Although the present assay system is focused on the determination of the target antigen molecules, it can be easily extend to the detection of other antigens or biocompounds. Moreover, the potential of this method for application is a simple and efficient diagnostic strategy for immunoassays.

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