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Piezoelectric immunosensor based on gold nanoparticles capped with mixed self-assembled monolayer for detection of carcinoembryonic antigen

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ABSTRACT

It is very important for a piezoelectric immunosensor to increase specific binding and decrease nonspecific adsorption. This study presents the development of such a piezoelectric immunosensor for the detection of carcinoembryonic antigen. An AT-cut quartz crystal's Au electrode surface was first modified with homogenous self-assembled monolayer of cysteamine (CE). Gold nanoparticles capped with mixed self-assembled monolayer of CE and MH (6-mercapto-1-haxanol) were then attached to the CE monolayer via glutaraldehyde (GA). Antibodies were immobilized onto a mixed self-assembled monolayer of CE and MH with GA as a reactive intermediate too. The binding of target antigens onto the immobilized antibodies decreased the sensor's resonant frequency, and the frequency shift was correlated to the antigen concentration. The stepwise assembly of the immunosensor was characterized by means of cyclic voltammetry technique. This immunoassay was shown to be specific and sensitive, thus providing a viable alternative to carcinoembryonic antigen detection method.

1. Introduction

Carcinoembryonic antigen (CEA) is an important tumor marker responsible for clinical diagnosis of colorectal, pancreatic, gastric, and cervical carcinomas. Various immunoassays, such as enzyme-linked immunosorbent assay, radioimmunoassay, and chemiluminescence immunoassay (CLIA) have been developed for detecting CEA. However, all these techniques are time-consuming, expensive and/or requirement for sample pretreatment and concentration. So it is still significant and necessary to explore some simple, sensitive, and low-cost methods for the detection of carcinoembryonic antigen. Piezoelectric quartz crystal (PQC) devices have been attracting more and more attention for years, for they are portable, simple, cost-effective, and suitable for real-time monitoring biospecific interactions between antigen and antibody with high sensitivity and selectivity.

Antibody immobilization is a vital part in development of a PQC immunosensor. The immobilization process must preserve the biological activity of the antibody, a more efficient binding and as well as reduce the nonspecific adsorption simultaneously. Current immobilization methods are mainly based on silanized layer [1], polymer membrane [2], Langmuir–Blodgett film [3], Protein A [4,5] self-assembled monolayer (SAM) [6–8] and nano-materials [9]. Of them, the SAM technique offers one of the simplest ways to provide a reproducible, ultrathin and well-ordered layer, however, pure SAM may decrease capture efficiency and specificity due to high density of terminal functional groups [10,11]. Recently, to overcome limitations associated with pure SAM, mixed SAMs resulting from the co-adsorption of two different thiols (i.e., mixed SAMs) have been tried to promote protein adsorption as a result of multiple chemical functionalities on the surfaces and decrease steric hindrance around the functional tails [12]. For example, Perez-Luna and his fellows fabricated the mixed SAMs of biotin-terminated thiol and 11-hydroxy-1-undecanethiol on a gold surface for improving specific binding and eliminating nonspecific adsorption of wild type streptavidin and streptavidin mutants [13]. Using 11-hydroxy-1-undecanethiol, Dubrovsky and his co-workers also increased specific binding and controlled the nonspecific adsorption of protein on the surface of the gold-coated silica gel [14]. Unfortunately, besides unavailability and high price of long-chain thiols, affinity interfaces made up of long-chain thiols are of relatively high viscoelasticity, which limits PQC application to the precise mass detection of biological materials in a liquid phase, because the Sauerbrey equation is derived from the assumption that the attached mass should be rigidly and strongly connected to the resonator [15]. Furthermore, these mixed SAMs assembled onto plane electrodes cannot supply enough functional groups to binding the probe molecules.

To cope with these problems, this paper focuses on antibody immobilization by way of mixed SAMs composed of short-chain thiols, which are assembled on gold nanoparticles (GNPs) with three-dimensional structure. In this strategy, cysteamine (CE) and 6-mercapto-1-haxanol (MH) were co-assembled on the surface of nanoparticles, therefore resulting in mixed-SAM-capped GNPs. Then,

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the mixed-SAM-capped GNPs were attached to gold electrode formally modified with cysteamine monolayer via glutaraldehyde (GA). MH functions as the "dilution reagent" to control the density of reactive groups on the surface, which increase the capture efficiency and minimize nonspecific adsorption. CE was utilized to introduce functional groups (–NH_2), which can provide reaction sites for covalently bonding to glutaradehyde. Antibodies were then immobilized through Schiff base via glutaradehyde cross-linking. A specific binding occurs between the immobilized antibodies and antigens. The whole process can be succinctly shown in Fig. 1.

2. Experimental details

2.1. Chemicals

Cysteamine, 6-mercapto-1-haxanol and glutaraldehyde were obtained from Sigma-Aldrich. $HAuCl_4 \cdot 4H_2O$ was purchased from Shanghai Chemical Reagents (Shanghai, China). Bovine serum albumin (BSA) and human complement C_3 (C_3) were bought from Beijing Dingguo Biological technology Company (Beijing, China). Anti-CEA antibody was purchased from Zhongshan Biotechnology Company (Beijing, China). Purified CEA antigen of human serum and serum of cancer patients were provided by Hunan Provincial Tumor Hospital. Phosphate-buffered saline solution (PBS, pH 7.0) was prepared using 0.01 M Na_2HPO_4 and 0.01 M KH_2PO_4. All other reagents were of analytical grade. Double distilled water was used throughout the experiments.

2.2. Apparatus

Electrochemical analysis was measured with a CHI760b electrochemistry working station (Jiangsu Chenhua Instruments, China). Cyclic voltammetry (CV) experiments were performed in a conventional three-electrode cell including a Pt electrode as counter electrode, a saturated calomel electrode as reference electrode and a modified Au electrode as working electrode. All electrochemical measurements were performed in the presence of 10 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) mixture, as a redox probe.

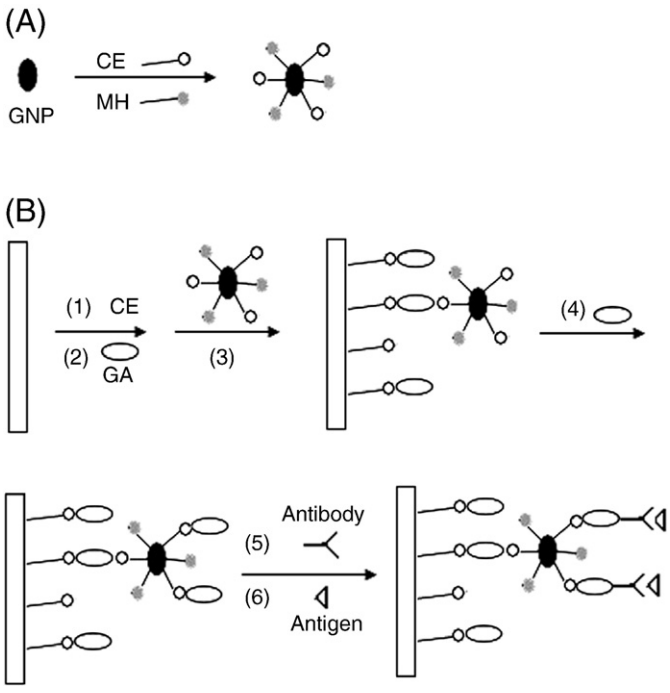

Fig. 1. Schematic illustration of the proposed immunosensor.

The piezoelectric quartz crystal (AT-cut, 9 MHz, gold electrode) was obtained from Chenxing Radio Equipments (Beijing, China). In order to stabilize the frequency in the solution, one side of the PQC was sealed with an O-ring of silicone rubber covered by a plastic plate forming an air compartment isolated from aqueous solution. The analysis of immobilization quantity of thin film, antibodies, antigens and nonspecific binding was performed by mounting the prepared PQC in a laboratory-made cell in which 3 mL of PBS was added. A laboratory-made transistor–transistor logic-integrated circuit was designed to drive the PQC at its resonance frequency. The resonant frequency was monitored with a high frequency counter (Model FC 1250, Wellstar). According to Sauerbrey equation, the frequency decrease is related to the mass change of biomoleculars immobilized on the surface of PQC.

2.3. Synthesis of Au nanoparticles

Various sizes of gold nanoparticles (10, 16, 25, and 30 nm) were prepared according to reference [16]. The mean diameter of the Au nanoparticles was determined using transmission electron microscopy (figures not shown).

2.4. Preparation of mixed-SAM-capped GNPs

We have studied a series of solution compositions for the preparation of mixed-SAM-capped GNP. In a typical reaction, 500 μL of CE (5 mM) and 100 μL MH (5 mM) were added into 5 mL of Au colloid. This process involved a co-adsorption of two different thiols. After 12 h, the reaction mixture was then purified by centrifuging and washing with ethanol and water for 3 times by repeating the resuspension and recentrifugation process to remove unbound CE and MH complexes. The procedure was shown in Fig. 1 A. The resulting precipitate was dispersed in distilled water again and stored at 4 °C.

2.5. Preparation of CEA immunosensor

Prior to the modification and measurement, each of the piezoelectric quartz crystal was cleaned in fresh piranha solution (70% H_2SO_4, 30% H_2O_2, v/v) followed by rinsing with water. The pretreated crystals were immersed in a solution of 5 mM cysteamine for 4 h to form a SAM. After rinsing with ethanol and water, 30 μL of GA was added onto the surface of crystal and incubated for 1 h at 37 °C. After rinsing with water and drying, the CE/GA-modified crystals were further immersed in a solution of mixed-SAM-capped GNPs for 6 h at 4 °C. After rinsing with water and drying, 30 μL of GA was added onto the surface of crystal and incubated for 1 h at 37 °C again. After rinsing with water and drying, 30 μL of anti-CEA antibody solution diluted with PBS (v/v = 1/2) was added onto the surface of crystal and incubated for 30 min at 37 °C. The excess antibodies were removed by rinsing with PBS. Then, the crystals were dried in air, and finally the sensor was ready. Before use, the sensors were stored at 4 °C.

2.6. Measurement procedure

The prepared PQC immunosensor were inserted into the reaction cell containing 3.0 mL of buffer solution (PBS, pH 7.0). With gentle stirring, the value of frequency was recorded when it reached stabilization (F_1). After the crystal was rinsed with water and dried in air, 30 μL of CEA antigen solution or serum of cancer patients with various concentrations was dropped onto the surface of PQC, incubated for 30 min at 37 °C and washed with PBS three times followed by being put into reaction cell. The value of frequency was recorded again when it reached stabilization (F_2). The ΔF ($\Delta F = F_2 - F_1$) responds to the frequency of immunoreaction and is correlated to the antigen concentration.