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Magnetic nanoparticle enhanced surface plasmon resonance sensor for estradiol analysis

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ABSTRACT

An enhanced surface plasmon resonance (SPR) biosensor system using magnetic nanoparticles as amplifying element was developed for the real-time determination of estradiol (E_2) based on indirect competition method. The anti-estradiol monoclonal antibody (E_2-mAb) was conjugated with the magnetic nanoparticles (MNPs) via protein A for signal amplification to improve the detection sensitivity. The chitosan, spin-coated on the sensor chip surface, was used to immobilize the antigen (E_2-BSA) by glutaraldehyde. The chitosan could significantly improve the performance and stability of the immobilized E_2-BSA. During the detection, E_2-BSA competed with E_2 in samples for binding with the E_2-mAb-MNPs conjugates. Then the SPR response decreased in the presence of E_2 because E_2 prevented the combination of E_2-mAb-MNPs conjugates and E_2-BSA. In other words, the response of SPR sensor was inversely proportional to E_2 concentration. Assay parameters, such as the amount of antibody and MNPs, the amount of immobilized E_2-BSA and E_2-mAb-MNPs concentration, were optimized in detail and E_2-spiked milk samples were detected. A good linear relationship was obtained between inhibition and lgC(E_2) ranging from 1.95 to 2000 ng/mL and the limit of detection (LOD) was 0.81 ng/mL. The result implied that the detection sensitivity was improved compared with the traditionnal sensor without magnetic nanoparticles. Meanwhile, an ELISA method was conducted to detect E_2 in milk as comparation with the enhanced SPR method, and the results indicated that these two methods had good consistency. The present study demonstrated a presumable general way and the enhanced sensors possessed a promising application for detection of various kinds of small molecules.

1. Introduction

Estradiol (E_2), one of endocrine disrupting chemicals (EDCs) [1], is the natural female sex steroid hormones [2]. As E_2 is used widely as growth promoting agents to cattle to increase the weight gain recently, excessive E_2 causes extra residue in animal food like milk [3] and come into human body. Excessive E_2 residue will cause endocrine and reproductive effects to human body, such as endometriosis, ovarian cancer, uterine fibroids in female groups and testicular cancer, inferior quantity and quality of sperm in male group [4,5]. Therefore, it becomes quite significant and urgent to detect E_2 effectively and specifically considering the issue of food safety.

So far, a variety of analytical methods has already been established to detect E_2 in foods such as immunological methods, chemiluminescence [6], gas chromatography/mass spectrography (GC/MS) [7,8], liquid chromatography/mass spectrometry (LC/MS) [9]. F. Valentini et al. used the enzyme-linked immunosorbent assay (ELISA) to detecte E_2 and the detecting limits were 1×10^{-7} M [10]. However, these detection methods required cumbersome procedures and complex pretreated processes,12 which were not suitable for rapid and real-time monitoring the residue of estradiol in food samples. In order to solve these tasks, it was imperative to develop a route for effective and rapid detection of trace E_2 with sensitivity and selectivity.

Surface plasmon resonance (SPR) sensor is one of the most promising sensors for trace detection in biomedical applications [11] due to its outstanding attributes such as rapid and real-time

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dynamic monitoring determination [12]. The sensor is an optical device which can precisely detect the changes of refractive index owing to the interaction between the sensor chip surface and the analytes [13]. In view of the advantages of SPR for biosensors, SPR has attracted much attention in applications in medical diagnostics [14,15], agro-food sector [16,17], virus monitoring [18], and genotype analyzing [19].

In order to improve the biocompatibility of Au chip and biological components like DNA, antibodies and enzymes, special materials and processes are necessary. Several approaches toward this goal have been demonstrated based on the use of self-assembled monolayers or surface-immobilized polymers [20–22]. The chitosan is one of the most interesting biopolymer for interfacing biology and biosensors due to its advantages like great film-forming ability, nontoxicity, mechanical strength, biocompatibility [23,24] and reduction of nonspecific adsorption [25]. Chitosan is a linear polysaccharide which consists of N-acetyl-D-glucosamineand and D-glucosamine linked by β-D (1 → 4), which can be obtained by deacetylation of chitin [26]. The unique propertie make it be easily handled in immobilizing biomaterials and construct biosensors [27]. Owing to its pH dependent solubility, it forms stable films on chip surface under neutral and basic pH conditions. Meanwhile its rich amine groups serve for covalent attachment of biomolecules.

The SPR sensor is highly sensitive to changes in the effective refractive index or the thickness of the test medium in the vicinity of the metal surface [28]. However, it is difficult to directly detect analytes at extremely dilute concentration (less than 1 pM) or with small molecular weight (less than 8 kDa) [29–31]. To overcome the challenge, numerous references have demonstrated that nanoparticles could be used as amplification regent which could improve the sensitivity owing to the large weight of nanoparticles, including Au NPs [32–34], Ag NPs [35], SiO_2 NPs [36], Pd NPs [37], and Pt NPs [38]. In terms of the high refractive index and the high weight of magnetic nanoparticles (MNPs) [39], it is possible to apply MNPs for enhanced SPR detection. Also, MNPs have large specific surface areas thereby allowing to conveniently conjugate biological samples like DNA and antibodies on their surface. All these outstanding characteristics make MNPs as promising candidates for labeling and signal enhancement.

In this work, we reported the development of an MNPs enhanced SPR immunosensor for the quantification of E_2 by indirect competition method. The chitosan was spin-coated on Au chip followed by an immobilization of E_2-BSA through glutaraldehyde. Then the mixture of E_2 and a certain concentration of conjugates of MNPs and anti-E_2 monoclonal antibody (E_2-mAb-MNPs) were injected into the cell. E_2-BSA competed with E_2 for binding to the E_2-mAb-MNPs conjugates. The less concentration of E_2 was, the more conjugates bound on the chip, which caused more SPR signal response. Assay parameters were optimized and the detecting specificity was investigated. Milk samples were detected as real samples using the enhanced SPR and the ELISA method, respectively, and the results indicated well consistency. The results demonstrated that the simple and sensitive SPR immunosensor represented a feasible tool for E_2 detection in milk samples.

2. Experimental

2.1. Reagent and materials

Anti-17-Estradiol monoclonal antibody (E_2-mAb, 2.5 mg/mL) was purchased from Abcam Inc, 17-Estradiol-BSA (E_2-BSA) were prepared in our laboratory [40]. E_2 and chitosan (average M.W 100,000–3,000,000) and glutaraldehyde were purchased from J&K SCIENTIFIC. Sodium dodecyl sulfate (SDS) was obtained from Sigma-Aldrich. Nanomag-D spio 20 nm nanoparticles coated by dextran matrix and protein A on the surface (MNPs) in 0.1 mol/L PBS buffer (pH 7.4, 5 mg/mL) were obtained from micromod Partikeltechnologie GmbH (Rostock, Germany). All reagents were of analytical grade and used without further purification, and ultrapure water was used throughout the experiments.

E_2-mAb was dissolved with 0.01 M phosphate buffer solution (PBS, pH 7.4). E_2-BSA was dissolved with 0.01 M sodium acetate buffer solution (NaAc-HAc, pH 4.5). E_2 was dissolved with 0.01 M PBS containing 5% methanol. Blocking buffer solution was 1 M ethanolamine (pH 8.5). Chitosan was dissolved with 2% (v/v) acetic acid solution.

2.2. Apparatus

The SPR measurements were performed on Autolab ESPRIT SPR (Eco Chemie B.V., Netherlands). Thoroughly degassed 0.01 M PBS (pH 7.4) buffer was used as the carrier solution. Chitosan film immobilized on the SPR chip surface was prepared by spin coating station (SC-1B, BeiJing Jinshengweina Technology Co., Ltd).

2.3. Functionalization of the SPR sensor chip

Initially, the chip was soaked in a piranha solution (H_2SO_4/H_2O = 7:3, v/v) in two hours to remove all organic substances and make the Au chip clean [41]. Then the chip was rinsed with plenty of water and ethanol, and then dried by nitrogen. Spin-coating method was employed to fabricate the chitosan film. The spin-coating was performed at 4000 rpm for 35 s and 100 μL chitosan solution (chitosan was dissolved in 2% Hac (v/v)) was anchored on the Au chip. After that, the chip was dried in air at the room temperature for 12 h. Finally, the chip was placed on the prism with the index matching oil and covered with the cuvette. After obtained the stable baseline, 50 μL 5% glutaraldehyde solution was injected into SPR cell to activate amino groups of chitosan for 10 min. Then 50 μL E_2-BSA was injected into the cell for the purpose of immobilization for 20 min. Then nonspecific adsorption was washed with 50 μL PBS. At last, blocking solution was injected to block unbound sites for 10 min.

2.4. Preparation of E_2-mAb-MNPs

E_2-mAb was conjugated with 20 nm spio nanoparticles via protein A which could bind the Fc fragment of E_2-mAb without affecting the specific binding between E_2-BSA and E_2-mAb. MNPs and E_2-mAb were diluted with PBST (PBS contained 5% tween 20) respectively. 100 μL E_2-mAb were mixed with 100 μL MNPs and the mixture was incubated with continuous shaking slowly at room temperature in 30 min. The mixture was then centrifuged at 15,000 rpm for 15 min. The supernatant fluid was collected for SPR analysis, and the precipitation, the conjugates (E_2-mAb-MNPs), was diluted with 200 μL PBS and stored in 4 °C.

2.5. Immunoassay

A schematic diagram of the experimental procedure is shown in Fig. 1. E_2 was dissolved in 0.1 mol/L PBS containing 5% methanol. The E_2 detection was performed in the form of an indirect competition assay using the E_2-mAb-MNPs as amplificatied material. The same conecentration of E_2-mAb-MNPs and different concentrations of E_2 (10,000, 5000, 2000, 1000, 500, 125, 62.5, 31.25, 7.81, 3.91, 1.95, 0.98, 0.49 ng/mL) was mixed with the same volume for 30 min at room temperature and the mixing solution was injected into SPR cell. The E_2-BSA and E_2 molecules competed for E_2-mAb and the amount of E_2-mAb-MNPs coupled with the E_2-BSA was monitored as a shift of the SPR angle. Consequently, the shift in