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Fluorescent aptasensor for 17β-estradiol determination based on gold nanoparticles quenching the fluorescence of Rhodamine B

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ABSTRACT

In this paper, we developed a fluorescent aptasensor for 17β-estradiol (E2) determination in aqueous solution using label-free E2-specific aptamer, gold nanoparticles (AuNPs) and Rhodamine B (RhoB) as sensing probe, fluorescent quencher and fluorescent indicator respectively. In the absence of E2, AuNPs were wrapped by E2 aptamer and maintained dispersed in NaCl solution basically. These dispersed AuNPs could effectively impair the originally high fluorescence of RhoB. Contrarily, in the presence of E2, E2 aptamer could specifically combine with E2 to form E2-aptamer complex, so the AuNPs were released by E2 aptamer and aggregated under the influence of NaCl. The aggregated AuNPs have a weak influence on RhoB fluorescence. Therefore, the E2 concentration can be determined by the change of fluorescence intensity of RhoB. This fluorescent assay has a detection limit as low as 0.48 nM, a linear range from 0.48 to 200 nM, and high selectivity over other disrupting chemicals. It was applied to determine E2 in water samples with recoveries in the range of 94.3–111.7%. The fluorescent aptasensor holds great potential for E2 detection in environmental water samples.

1. Introduction

17β-estradiol (E2) is a major estrogen in human and domestic animals, being essential for the development of female reproductive system and maintenance of sexual characteristics [1]. As a typical endocrine disrupting chemical, E2 has received extensive attention from researchers because of their harmful effects on the environment and organism [2,3]. E2 can enter into surface water through discharges from domestic sewage effluents, industrial facility effluents, animal feeding operations and septic system [4]. In fact, many studies have reported that the concentrations of E2 in surface waters of diverse countries range from ng/L to μg/L levels [5–7]. Even a low concentration of E2 entering into the organism body via food chain could interfere with the normal endocrine function, further causing many deleterious effects [8,9]. For these reasons, the European Union has added E2 to a new "watch list" of emerging aquatic pollutants included in the Water Framework Directive recently [10]. So it is highly necessary to develop valid and robust methods for monitoring E2 in aqueous environment.

Several analytical methods for E2 determination have been established. Instrumental analysis methods, such as GC/MS or HPLC [11,12], require complex sample pretreatment and long analysis time despite their accuracy and sensitivity. Immunoassays are analytical technology based on the specific reaction of antigen and antibody, mainly including enzyme-linked immune sorbent assay, fluorescence immunoassay and immune chip measurement [13–15]. Immunoassays achieve the high affinity and specific molecular recognition. On the other hand, they have some other limitations, including relative instability, complex production and purification steps from animals or cell lines [16]. Electrochemical methods received much attention because of its good sensitivity and high economic benefits, but the aptamer usually needs to be modified and immobilized on the electrode [17,18]. In recent years, a new type of aptasensor based on biological technology, combined with sensing technology, nanotechnology and photoelectric technology has been developed.

Aptamer is a type of functional nucleic acids selected in vitro

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through Systematic Evolution of Ligands by EXponential enrichment (SELEX) [19,20]. The pattern of aptamer recognizing target is similar to the antibody. In addition to high affinity and specificity, aptamer has more advantages compared with antibody, embracing production automation, good stability, desirable biocompatibility and flexible chemical-modification [21]. Since E2-specific aptamer was screened out in 2007 firstly through SELEX [22], it has been widely used as recognition element and applied in aptamer-based biosensors for E2 detection. According to the difference of signal obtained, the aptasensors are divided into electrochemical, optical and mass-sensitive [21]. Some electrochemical aptasensors for femtomolar detection of E2 have been developed [23,24]. In optical aptasensor, fluorescence and colorimetry are the two most popular methods [25,26]. Gold nanoparticles (AuNPs) were regarded as the most desired nanoparticles widely used in optical aptasensors because of their high extinction coefficients and distinct size-dependent optical properties [27]. Unmodified AuNPs as colorimetric indicator have been given outstanding attention for their simplicity, high sensitivity, and potential high-throughput analysis [28,29]. Besides, AuNPs have been found have a pronounced effect on the photoluminescence of dyes such as Rhodamine B (RhoB) and Rhodamine 6G, so some fluorescence aptasensors using AuNPs as "super quenchers" have been developed [30–33].

RhoB is a kind of synthetic dye with the feature of strong fluorescence, photostability, and water-solubility [34]. Zhang et al. have demonstrated that when RhoB absorbed to the surface of AuNPs through electrostatic interaction, dispersed AuNPs could greatly quench the fluorescence of RhoB, while aggregated AuNPs showed a weaker quenching ability [32]. The difference of quenching ability between dispersed and aggregated AuNPs probably lie in the surface-to-volume ratio of AuNPs and the fluorescence inner filter effect of AuNPs [32–34]. In this study, on the basis of the classical colorimetry and quenching properties of AuNPs, RhoB was introduced to convert the colorimetric signal into fluorescent signal for E2 detection in aqueous solution with the purpose of improving the sensitivity to a certain extent [35].

2. Materials and methods

2.1. Chemicals and apparatus

The E2-specific aptamer was synthesized by Sangon Biotechnology Inc. (Shanghai, China) [22]. Its sequence is 5′-GCTTCCAGCTTATTGAATTACACGCAGAGGGTAGCGGCTCTGCGCATTC AATTGCTGCGCGCTGAAGCGCGGAAGC-3′ (76-mer). E2, RhoB, bisphenol A (BPA), *o,p′*-dichlorodiphenyltrichloroethane (*o,p′*-DDT), tetracycline (TET), 7-aminocephalosporanic acid (7-ACA) were obtained from Aladdin Biotechnology Inc. (Shanghai, China). Sodium citrate ($C_6H_5Na_3O_7$) and sodium chloride (NaCl) were purchased from Beijing Chemical Reagents Company (Beijing, China). Chloroauric acid ($HAuCl_4 \cdot 4H_2O$), 3-(N-morpholino) propanesulfonic acid (MOPS), progesterone (PRG), diethylstilbestrol (DES) were obtained from Sangon Biotechnology Inc. (Shanghai, China). Unless otherwise mentioned, all other reagents were analytical grade and used without further purification or treatment. MOPS buffer solution (10 mM, pH 7.0) was used in all experiments. Ultrapure water (Milli-Q plus, Millipore Inc., Bedford, MA) was used throughout the experiment for aqueous solution preparation.

The 96-well microplate was purchased from Thermo Fisher Scientific Inc. (Nunclon, Denmark). F-4500 fluorescence spectrophotometer (Hitachi, Japan) was used to record the fluorescence intensity, with an excited slit of 10 nm, emission slit of 2.5 nm, photomultiplier tube voltage of 700 V and excitation wavelength of 520 nm. And a transmission electron microscope (TEM) JEM-2010HT (Hitachi, Japan) was used to observe the images of AuNPs. An Infinite M200 Pro microplate spectrophotometer (Tecan Austria GmbH, Salzburg, Austria) was employed to measure the absorbance of reaction solutions. Thermostatic incubating device (Eppendorf, China) was used to maintain the incubating temperatures at 25 °C.

2.2. Preparation of AuNPs

The AuNPs were synthesized by sodium citrate reduction of $HAuCl_4 \cdot 4H_2O$ following a literature procedure [28]. First, all glassware used in this experiment were cleaned by freshly prepared 3:1 (v/v) HNO_3/HCl and rinsed thoroughly with ultrapure water. Then, a sodium citrate solution (1% (w/v), 10.5 mL) was rapidly added to a boiled solution of $HAuCl_4 \cdot 4H_2O$ (100 mL, 0.03% (w/w)) and vigorous stirred for 30 min. After that, the heater of the magnetic stirrer was not turned off until the mix solution appeared wine red steadily, and stirred for another 20 min. Last, the resulting wine-red solution was cooled to room temperature and filtered, then stored in dark glass bottle at 4 °C for further use.

2.3. Optimization of detection conditions

The volume of each sensing system was set as 500 μL and the volume of AuNPs added to each sensing system was fixed at 100 μL. The original concentration of NaCl solution, E2-specific aptamer and RhoB used was 2 M, 1 μM and 200 μM respectively. The absorbance values at 520 nm (*A*520) and 650 nm (*A*650) were measured because these two values could represent the relative amount of the free and aggregated gold nanoparticles, respectively. The absorbance ratio $A = A650/A520$ was calculated to evaluate the aggregation degree of AuNPs.

2.3.1. Optimization of NaCl concentration

When optimizing the NaCl concentration, 100 μL AuNPs was added into centrifugation tubes containing 400, 395, 390, 385, 380, 375, 370, 365, 360, 355, 350 μL MOPS buffer (10 mM, pH 7.0) respectively and mixed thoroughly. Then, different volumes of original NaCl solution (0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 μl, 2 M) were put in the tubes severally to attain a total volume of 500 μL. The final concentration of NaCl solution was 0, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200 mM respectively. After incubating for 5 min, the absorbance from 400 to 800 nm was measured by microplate spectrophotometer M200 Pro. The NaCl concentration that resulted in a maximum *A*650/*A*520 value was chosen.

2.3.2. Optimization of E2-specific aptamer concentration

In order to optimized the concentration of E2-specific aptamer, different concentrations of aptamer (0, 5, 10, 15, 20, 25, 30, 40, 50, 60 nM) were incubated with 100 μL AuNPs in MOPS buffer for 15 min. Next, the optimized concentration of NaCl was added into all groups and mixed thoroughly. Finally the absorbance was measured and E2 aptamer concentration that resulted in a minimum *A*650/*A*520 value was chosen.

2.3.3. Optimization of RhoB concentration

The RhoB concentration was optimized by comparing the fluorescent intensity of the sensing system in the absence and in the presence of E2. At first, MOPS, the optimized aptamer and AuNPs solution were added into the 1.5 mL centrifugation tubes and mixed thoroughly. After incubating 20 min, E2 solutions (25 μL, 10 μM) was added in the tubes of the experimental groups and incubated for another 20 min, then optimized NaCl and RhoB with different concentrations (2, 4, 6, 8, 10, 12, 14 μM) were added. The blank experiments were carried out by replacing E2 solution with ultrapure water. Finally, F_0 and F that represented the fluorescent