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Colorimetric aptasensor for progesterone detection based on surfactant-induced aggregation of gold nanoparticles

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ABSTRACT

This paper proposes an aptasensor for progesterone (P4) detection in human serum and urine based on the aggregating behavior of gold nanoparticles (AuNPs) controlled by the interactions among P4-binding aptamer, target P4 and cationic surfactant hexadecyltrimethylammonium bromide (CTAB). The aptamer can form an aptamer-P4 complex with P4, leaving CTAB free to aggregate AuNPs in this aptasensor. Thus, the sensing solution will turn from red (520 nm) to blue (650 nm) in the presence of P4 because P4 aptamers are used up firstly owing to the formation of an aptamer-P4 complex, leaving CTAB free to aggregate AuNPs. However, in the absence of P4, CTAB combines with aptamers so that AuNPs still remain dispersed. Therefore, this assay makes it possible to detect P4 not only by absorbance measurement but also through naked eyes. By monitoring the variation of absorbance and color, a CTAB-induced colorimetric assay for P4 detection was established with a detection limit of 0.89 nM. Besides, the absorbance ratio $A650/A520$ has a linear correlation with the P4 concentration of 0.89–500 nM. Due to the excellent recoveries in serum and urine, this biosensor has great potential with respect to the visual and instrumental detection of P4 in biological fluids.

1. Introduction

Progesterone (P4) is a steroid hormone that is essential for the development of mammary tissue and maintenance of pregnancy. It also helps female regulate the menstrual cycle, and plays an important role in menopausal hormone therapy and oral contraceptives [1,2]. P4 is an indispensable for human body and the concentration varies in different period of life. For example, the concentrations of serum progesterone in adult females are normally in the range of ~0.48–~79.5 nM, while the P4 concentrations in pregnant women can rise to ~731 nM [3]. If P4 concentration is below the normal level, people need to take P4 supplements. However, when the concentration of P4 is over the normal level, P4 can be toxic and have carcinogenic effects [4]. So it is crucial to monitor and control P4 concentration in an appropriate range. Yet, P4 detection in biological matrices is challenging since the concentrations are around the ng mL^{-1} (1 ng mL^{-1} = 0.325 nM) level or even lower [5]. Thus, more sensitive and effective assay is required.

Although many analytical methods have been developed to monitor P4, there are only two kinds of methods commonly used which is immunoassays (radioimmunoassay, enzyme-linked immunosorbent assay, electrochemical immunoassay, etc.) and chromatographic methods (high performance liquid chromatography, liquid chromatography-mass spectrometry, gas chromatography-mass spectrometry et al.) [6,7]. These methods are sensitive but they require expensive equipments and complicated performance procedures, or some suffer from poor specificity, accuracy and reproducibility because of the cross-reaction and batch-to-batch variation of antibodies [8]. Aptamer was first selected and named in 1990 [9,10]. Since then, aptamers of various targets (metal ions, proteins, antibiotics, etc.) have been selected and applied to their target detection [11–20]. There is intermolecular interaction between the aptamer and its target so that they can bind to each other with high selectivity and affinity [14]. Thus, aptamers have been applied to clinical, medical monitoring and food analysis [21]. Recently, some researchers successfully selected P4 aptamer and developed an electrochemical aptasensor with a

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detection limit of 2.86 nM [12]. And based on this breakthrough, our group had built a NaCl-induced colorimetric aptasensor which obtained a LOD of 2.62 nM [22].

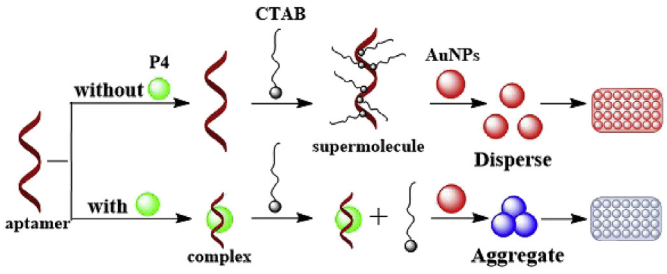
Gold nanoparticle (AuNP) is superior material for signal transduction due to its aggregation behavior and has been frequently used in the design of biosensors [23–28]. AuNPs alone are dispersed and appear red in solution. The aggregation of AuNPs will bring about an increase in the size of particles in solution, leading to a rise in the absorbance within a certain range and a color change to blue.

Hexadecyltrimethylammonium bromide (CTAB) possesses two effective features in this biosensor: CTAB can not only aggregate AuNPs but also bind to DNA, owing to its positive charge and the electrostatic attraction [29–31]. As a cationic surfactant, CTAB has also been applied in the isolation of genomic DNA from plants samples. Futhermore, the binding of CTAB to ssDNA and dsDNA are different: ssDNA-CTAB complexes are formed with cubic nanostructures, while dsDNA forms hexagonal nanostructures with CTAB [23,32]. The principle of the colorimetric biosensor under ideal conditions was outlined in Scheme 1. In the absence of P4, P4 aptamers are free to form some supramolecule with CTAB, so that the AuNPs maintain dispersed. However, the sensing solution will turn from red to blue in the presence of P4 because P4 aptamer are used up firstly owing to the formation of an aptamer-P4 complex, leaving CTAB free to cause the aggregation of AuNPs. Besides, the absorbance ratio *A*650/*A*520 has a linear correlation with the concentration of P4 in a certain range. Therefore, this assay makes it possible to detect P4 in biological fluids not only by absorbance measurement but also through naked eyes.

2. Materials and methods

2.1. Chemicals and materials

CTAB, diethylstilbestrol (DES), ampicillin (AMP), thiamphenine (THI), aztreonam (AZT), were obtained from Sigma-Aldrich (St. Louis, MO, USA; www.sigmaaldrich.com). P4, 17β-estradiol (E2), estriol (E3), bisphenol A (BPA), sodium citrate, $HAuCl_4 \cdot 4H_2O$, NaOH, 3-(N-Morpholino) propanesulfonic acid (MOPS, 10 mM, pH 8.0), were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China; shreagent.lookchem.com). Synthetic human urine and serum were purchased from BIOMART.CN (www.biomart.cn). Unless otherwise mentioned, all other reagents were of analytical reagent grade and used without further purification. The 96-well microplates and centrifuge tubes were bought from Thermo Fisher Scientific Inc. (Nunclon, Denmark; www.thermofisher.com/cn/zh/home.html). The ultrapure water used in all experiments and aqueous solution preparation was purified by a Milli-Q Advantage A10 System (Millipore Inc., Bedford, MA, USA).



Scheme 1. Schematic diagram of the colorimetric aptasensor for progesterone (P4) detection based on hexadecyltrimethylammonium bromide (CTAB)-induced aggregation of AuNPs.

The sequence of P4 aptamer is 5′-GCATCACACACCGATACTCACCCGCCTGATTAACATTAGCCCACCGCCCACCCCCGCTGC-3′ (60-mer) [12], synthesized by Sangon Biotechnology Inc. (Shanghai, China; www.sangon.com). The aptamer was dissolved in ultrapure water and stored at 4 °C before use.

2.2. Instrumentation

Colorimetric assays were recorded on an Infinite M200 Pro microplate spectrophotometer (Tecan Austria GmbH, Salzburg, Austria). A thermostat (Eppendorf, Hamburg, Germany) was used for incubation. A circular dichroism spectroscopy (CD; J-815, Jasco, Japan) was employed to characterize the structural changes of P4 aptamer. The average diameter of AuNPs was measured on a Zetasizer Nano S (Malvern, England).

2.3. Preparation of AuNPs

AuNPs were prepared by sodium citrate reduction of $HAuCl_4 \cdot 4H_2O$, based on a previous study [24]. First, the boiled solution of $HAuCl_4 \cdot 4H_2O$ (100 mL, 0.03% (w/w) was added with 3.5 mL of 1% (w/v) sodium citrate solution, then continue to be boiled and stirred for 30 min. Within that period, the color of solution changed gradually from light grey, blue, purple, and to wine red finally. The mixture was boiled for another 10 min and stirred until it was cooled to room temperature. At last, the cooled solution was filtered by 0.2 μm ultrafiltration membranes (HP134, Sangon Biotechnology Inc.), and then stored in a brown glass bottle at 4 °C for further use.

2.4. Characterization of the interactions among aptamer, P4, CTAB and AuNPs

The CD spectrum of 4 samples was tested: (a) 200 nM aptamer; (b) 200 nM aptamer + 200 nM P4; (c) 200 nM aptamer + 40 μM CTAB; (d) 200 nM aptamer + 200 nM P4 + 40 μM CTAB. Sample (a) was directly tested, sample (b) and (c) were tested after 20-min incubation. As for sample (d), CTAB was added after 20-min incubation of the aptamer and P4, then the CD spectrum was tested after another 20 min.

The dynamic light scattering (DLS) is to measure the average diameter of AuNPs in 4 different samples: (a) AuNPs; (b)

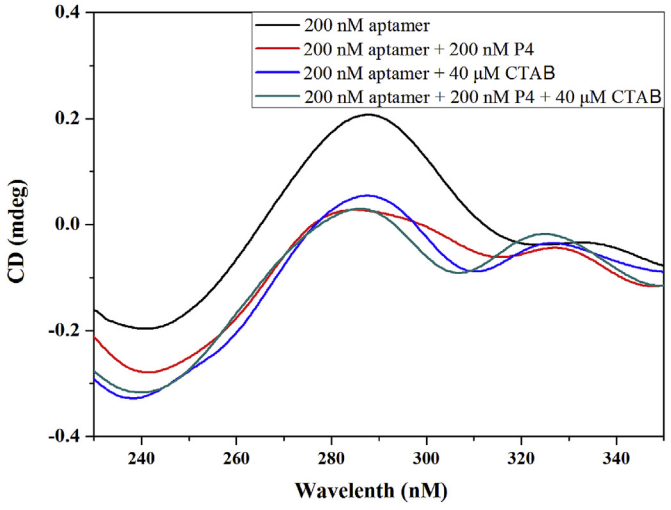


Fig. 1. Circular dichroism (CD) spectra of progesterone (P4) aptamer treated with P4 or hexadecyltrimethylammonium bromide (CTAB).