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RESEARCH PAPER

Efficient Detection of Environmental Estrogens Bisphenol A and Estradiol By Sensing System Based on AuNP-AuNP-UCNP Triple Structure

LI Qiao-Feng[1,2], REN Shu-Yue[2], WANG Yu[2], BAI Jia-Lei[2], PENG Yuan[2], NING Bao-An[2], LYU Quan-Jun[1], GAO Zhi-Xian[1,2,*]

[1] Department of Nutrition and Food Hygiene, College of Public Health, Zhengzhou University, Zhengzhou 540001, China;
[2] Tianjin Key Laboratory of Risk Assessment and Control Technology for Environment and Food Safety, Institute of Environmental and Operational Medicine, Academy of Military Medical Science, Academy of Military Science, Tianjin 300050, China

Abstract: A sensing system based on AuNP-AuNP-UCNP triple structure for efficient detection of dual targets bisphenol A and estradiol was constructed. In the preparation of triple structure, the gold nanoparticles (AuNPs) and upconversion nanoparticles ($NaYF_4$: Yb, Er, Gd, UCNPs) were synthesized and surface modified. Then, the two nanoparticles and their aptamers were connected to form two kinds of optical fluorescent probes. A nucleic acid sequence that matches with two aptamers was designed, rendering the probes to get close based on the principle of complementary base pairing. On the basis of this, a sensing system with triple structure was prepared, and its connecting effect was characterized by TEM. With this system, dual targets of bisphenol A and estradiol were detected efficiently and conveniently through quantitatively determination by fluorescence and UV spectrophotometer. At the reaction temperature 30 ºC and pH 7.8, this method exhibited good linear range for determination of bisphenol A and estradiol from 2 ng mL^{-1} to 200 ng mL^{-1} and from 10 ng mL^{-1} to 150 ng mL^{-1}, with the limits of detection of 0.2 ng mL^{-1} and 0.5 ng mL^{-1}, respectively. This sensing system with triple structure owned better specificity to structural and functional analogues, showed good repeatability and stability. What's more, this sensing system could be applied to actual water detection, with the recoveries of 86.1%–107.4%, and the relative standard deviation below 6.8%. This method shows promising applications in other environmental estrogens in water sample.

Key Words: Gold nanoparticles; Upconversion nanoparticle; Triple structure; Bisphenol A; Estradiol

1 Introduction

Environmental estrogen is a kind of chemical compound that interferes with the endocrine system and disrupts the stability and regulation of the human race[1]. Bisphenol A (BPA) and estradiol (E2) are two typical environmental estrogens which are reported frequently in the toxic or carcinogenic effects of such substances on human body[2–6]. There are mainly two conventional methods to detect BPA and E2. (1) Large-scale instruments, including high performance liquid chromatography (HPLC)[7–9], liquid chromatography-mass spectrometry (LC-MS)[10,11] and gas chromatography-mass spectrometry (GC-MS)[12], have some advantages such as good accuracy and reliability, but they could not meet the needs of rapid detection because of the expensive equipment, the complex sample pretreatment and the operation of professionals. (2) Immunoassay, including enzyme linked immunosorbent assay (ELISA)[13] and fluorescence immunoassay[14] which based on antigen antibody reaction, could guarantee the detection sensitivity and selectivity, with simple and convenient sample pretreatment. But the disadvantages including the non-specific

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*Corresponding author. E-mail: gaozhx@163.com
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binding of antibody and the environmental interference caused by the experimental process would lead to inaccuracy results. Therefore, it is urgent to develop a highly efficient, convenient and environment-friendly method for determination of environmental estrogen.

In recent years, more and more researches have been reported for rapid detection of biochemical detection by fluorescence sensor. Due to the high extinction coefficient, good optical properties and high fluorescence quenching efficiency[15,16], gold nanoparticles (AuNPs) are often used as optical nanoparticle probes in biochemical detection. With the in-depth knowledge of fluorescent nanomaterials, researchers found that the near infrared light excited upconversion nanoparticles ($NaYF_4$: Yb, Er UCNPs) possessing the advantages such as high signal-to-noise ratio, excellent chemical stability, little photobleaching and strong penetration capability were widely used in biomedical field[17,18]. Moreover, the emission spectra of UCNPs (maximum emission wavelength at 550 nm) overlapped with the absorption spectra of AuNPs (maximum absorption peak at 530 nm). Under certain conditions, the phenomenon of fluorescence resonance energy transfer (FRET)[19] occurred with UCNPs as the energy donor and AuNPs as the energy acceptor. Screened from synthetic oligonucleotide library by systematic evolution of ligands by exponential enrichment (SELEX), aptamer has high specificity to combine with DNA, RNA, proteins and other small molecules. Therefore, it was applied to the development of all kinds of biosensors[20,21]. At present, the fluorescence detection methods based on aptamers and FRET are constantly emerging[22,23], which provide a basis for rapid detection of small molecules.

In this study, AuNPs and $NaYF_4$: Yb, Er, Gd, UCNPs as two optical probes were connected with aptamers of BPA and E2, respectively, forming AuNP-AuNP-UCNP triple structure by a total complementary sequence linker (cDNA) so as to realize FRET. The schematic of the proposed method is shown in Fig.1. First, the triple structure was prepared, and then the analyte containing BPA and E2 was added to the system. The specific binding ability between aptamer and target made AuNPs and UCNPs connected with aptamer detach from the triple structure. Finally, the products were separated by centrifugation, and the fluorescence intensity of UCNPs in supernatant and UV absorbance intensity of AuNPs in precipitation were measured. On the basis of this, the efficient and time-saving detection for the dual targets of bisphenol A and estradiol were realized with the same system, and a sensitive, convenient and efficient sensing system for detecting environmental estrogen in small scale laboratory was established.

2 Experimental

2.1 Instruments and reagents

TU-1901 spectrometer (Shimadzu, Japan), F97-Pro fluorescence spectrophotometer (Shanghai, China) with an external 980 nm laser (Beijing, China), HT7700 transmission electron microscope (TEM, JEOL, Japan), TGL-16C centrifuge (Shanghai, China), ZWY-240 constant temperature culture oscillator (Shanghai, China), 98-II-B hotplate stirrer for round-bottom-flasks (Tianjin, China) and KQ-500E ultrasonic cleaner (Kunshan, China) were used in this study. E2, BPA, diethylstilbestrol (DES), nonylphenol (NP) and ractopamine (RAC) were purchased from Sigma-Aldrich. Dihydroxybiphenyl (BP), Bishydroxyphenylbutane (BPB), tetrabromobisphenol A (TBBPA) and 6F-bisphenol A (6F-BPA) were purchased from Shanghai Yuanye Biotechnology Co., Ltd. All the above reagents were prepared with methanol as reserve liquid. Ultrapure water (Millipore, Z18 MΩ cm) was prepared by a Milli-Q filtration system. E2 aptamer[24], BPA aptamer[25] and complementary sequence were synthesized by Sangon Biotech (Shanghai) Co., Ltd, as shown in Table 1.

Prior to use, the above nucleic acid sequences were dissolved in 100 nM PBS buffer (pH 7.4), heated to 95 ℃ for 5 min, and then slowly cooled to 4 ℃ for storage. The conditions of fluorescence measurement were as follows: the upconversion luminescence spectra was collected by F97-Pro fluorescence

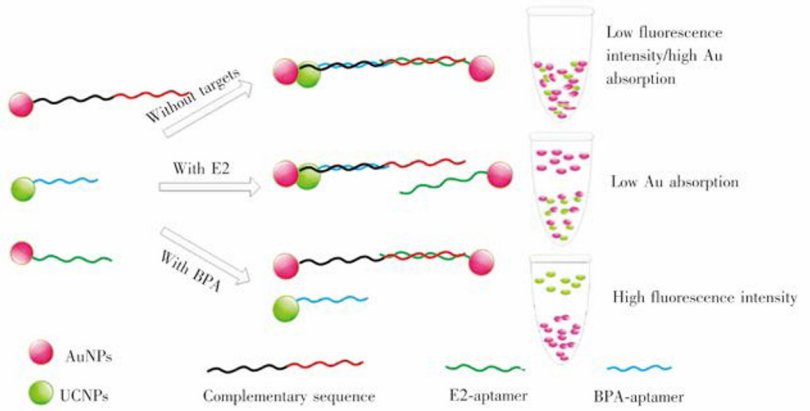

Fig.1 Schematic illustration of efficient detection of BPA and E2 by AuNP-AuNP-UCNP sensing system