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Turn-on fluorescent assay based on purification system via magnetic separation for highly sensitive probing of adenosine

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ABSTRACT

In this work, based on purifying the analysis system by magnetic separation, we constructed a turn-on fluorescent assay for highly sensitive detection of possible biomarkers, employing adenosine as the model target analyte. Aptamer labeled-carbon dots (Apt-CDs) was applied as fluorescence probes as well as selective recognition elements, while Fe_3O_4@polypyrrole (Fe_3O_4@PPY) was used as fluorescence quenching and magnetic separating materials for system purification. In absence of adenosine, the Apt-CDs were attached to the surface of PPY through π-π stacking and hydrophobic interaction and its fluorescence was quenched by Fe_3O_4@PPY. In presence of adenosine, the aptamer could specifically bind with adenosine to form a three dimensional Apt-CDs-adenosine complex and detach from PPY, leading to the fluorescence recovery of Apt-CDs. More importantly, with analysis system purification via twice convenient magnetic separations of free CDs and Apt-CDs-adenosine complex respectively, the detection limit could be reduced from 8 nmol L^{-1} to 0.15 nmol L^{-1}. This concept offers potential application for simple, rapid, cost-effective, and highly sensitive assay of biological samples.

1. Introduction

Fluorescence analysis has been intensively applied for the probing of different analytes in various reasearch fields such as clinical diagnosis, food safety and environmental monitoring due to their high sensitivity, high throughput and simple instrumentation [1–3]. Different fluorescence probes, e.g. fluorescence dyes, quantum dots and carbon dots (CDs) have been extensively utilized in fluorescence assays [4]. CDs with excellent optical properties have received extensive interests recently due to their exceptional advantages such as easy preparation, low toxicity, good biocompatibility, and favorable water solubility [5–7] Aptamers, single-stranded oligonucleotides selected in vitro, exhibit high affinity and specific binding to large-scale molecular targets in biosensor applications [8–10]. Thereby, the combination of CDs with aptamer produces a fluorescent assay with high sensitivity and selectivity.

To further improve selectivity and sensitivity of bioanalytical methods and allow reduction of time-consuming analysis, the introduction of fluorescence quenching materials was exploited. Polypyrrole (PPY) is one of the most widely used conducting polymers in fabrication of bio-sensing field due to its low cost, easy synthesis and excellent environmental stability [11–13]. Ramanavicius et al. used PPY as an efficient fluorescence quencher in fluorescence detection based immunosensors firstly [14]. Recently, PPY was used as an acceptor for the first time and the CdS QD as the donor in fluorescence assay by Hashemian et al. [15].

Fluorescence assay was seriously interfered by matrix in analysis system (the mixture that was used to detect fluorescence intensity), so high purification of analysis system was required to enhance the sensitivity. Thereby, the separation of free donors and acceptors species was of great significance to obtain pure analysis system in the "off-on" inhibition assay. However, to date, the separation of free donors from inhibition systems to purify the analysis system has been rarely reported in fluorescence assay. In this study, Fe_3O_4@PPY was applied not only to quench fluorescence more efficiently but also to purify the analysis system conveniently. For the first separation, free donors CDs were removed, leading to sharp decrease of background signal. For the second separation, scattering interference from acceptors was declined significantly by the separation of Apt-CDs combined with the target adenosine from the system. Based on twice magnetic separation, analysis system was purified effectively, suggesting the potential for the improvement of intensity recovery and sensitivity of the fluorescence assay.

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Adenosine plays a crucial role in multiple critical bioprocesses, and it is an essential component of many biological cofactors [16,17]. That is, adenosine could be considered as possible biomarkers for cancer, and also shows potential in early clinical diagnosis [15,18,19]. Thus, the great significance of monitoring adenosine at the preliminary stage of clinical diagnosis and treatment is self-evident. Herein, a facile and novel turn-on fluorescence assay based on CDs as a donor, aptamer as the recognition element and Fe_3O_4@PPY as an acceptor was fabricated for probing of adenosine. The introduction of target adenosine triggered the switch of the aptamer conformation and the detachment from the surface of PPY; thus, led to the fluorescence recovery of Apt-CDs quenched by Fe_3O_4@PPY. Meanwhile, the sensitivity could be further improved with the introduction of magnetic Fe_3O_4@PPY. The as-prepared fluorescence assay was applied to detect adenosine in biological samples, indicating its foreseeable prospect in the field of clinical diagnosis.

2. Experimental

2.1. Reagents and materials

Adenosine, guanosine, uridine, cytidine, $FeCl_2{\cdot}4H_2O$ and pyrrole were purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). $FeCl_3{\cdot}6H_2O$ and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Ammonium persulfate was purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China). Adenosine aptamer (sequence: 5′-NH2-$(CH_2)_6$-AGA GAA CCT GGG GGA GTA TTG CGG AGG AAG GT-3′) was synthesized by Sangon Biotechnology Co. Ltd. (Shanghai, China).

2.2. Apparatus

Fluorescence measurements were performed using a Hitachi F-4600 (Japan) fluorescence instrument. X-ray photoelectron spectroscopy (XPS) measurements were conducted by a PHI Quantera II spectrometer (Japan) and samples were freeze-dried before measurement. Transmission electron microscopy (TEM) and high-resolution transmission electron microscopy (HRTEM) images were recorded using JEM-2100 (HR) transmission electron microscopy (JEOL, Japan). FT-IR spectra were achieved on a TENSOR27 infrared scanner (Bruker, Germany). Fluorescence decay curves were recorded by a FM-4P-TCSPC transient fluorescence spectrometer (Horiba Jobin Yvon, U.K.). The zeta potential was determined by Zs90 dynamic light scattering (DLS) (Malvern, U.K.). The synthesis of Apt-CDs was confirmed by gel electrophoresis in 12% Native-PAGE gel with 0.5 × TBE buffer at 80 V for 50 min, and gel image was photographed by 3500R Gel Image System (Tanon, China).

2.3. Synthesis of Fe_3O_4@PPY NPs

Fe_3O_4 nanoparticles (NPs) were synthesized according to our previous report [20]. 4.72 g $FeCl_3{\cdot}6H_2O$ and 1.72 g $FeCl_2{\cdot}4H_2O$ were dissolved in 80 mL of water with nitrogen flow under vigorous stirring for 15 min. Then, Fe_3O_4 NPs were formed by adding 10 mL aqueous ammonia (27%) dropwise and rising the temperature to 80 °C. After 30 min rapid stir, the obtained black product were achieved by magnetic separation, washed by ethanol, deionized water successively, then dried in vacuum. Subsequently, 20 mg Fe_3O_4 NPs were dispersed in 12 mL H_2O, 30 μL pyrrole (99%) was added with stirring for 30 min. After that, 3 mL of 0.14 mol L^{-1} ammonium persulfate was dropped and incubated for 4 h at 0–5 °C. Subsequently, the Fe_3O_4@PPY NPs were obtained via magnetic separation and washed by ethanol, deionized water in sequence.

2.4. Preparation of adenosine aptamer labeled-CDs (Apt-CDs)

The CDs were prepared according to the procedure reported in the literature [21]. Briefly, by heating 2 g citric acid at 200 °C for 30 min with a heating mantle, the orange liquid was obtained. Under rapid stirring, the liquid was dropped into 100 mL NaOH solution (10 mg mL^{-1}). Then the aqueous solution of CDs was obtained by using NaOH to adjust pH value to 7.0.

The Apt-CDs were prepared via well-established carbodiimide method. 190 μL of 20 g L^{-1} EDC in 10 mM PBS and 200 μL of CDs solution were mixed together and sonicated for 30 min. After the addition of 10 μL of 100 μM adenosine aptamer, the mixture was further incubated for 4 h at 25 °C. Afterwards, the obtained Apt-CDs were refrigerated overnight for future use.

2.5. Detection of adenosine

For adenosine determination, 20 μL of Fe_3O_4@PPY (1 mg mL^{-1}) was added into 20 μL of above-mentioned Apt-CDs solution and incubated for 20 min to quench the fluorescence, then the free CDs was removed and the Apt-CDs- Fe_3O_4@PPY complex was redissolved after three washes. Subsequently, 20 μL of adenosine with various concentrations was introduced into the mixture and shaken continuously using a spinning mixer for 30 min at room temperature. Finally, the mixture was diluted to 100 μL by deionized water for fluorescence measurements. High sensitivity could be achieved with low-quenched and high-recovered fluorescence. Hence, the relative fluorescence intensity $[(F\text{-}F_0)/F_0]$ was calculated to evaluate the output signal, where F and F_0 represent the fluorescence intensities of the proposed assay after magnetic separation with or without target adenosine, respectively (Scheme 1). The direct measurement without magnetic separation was also shown in Scheme 1.

2.6. Analysis of serum sample

Serum samples of healthy individuals were obtained from Nanjing Brain Hospital and stored frozen when not in use. Serum samples were centrifuged for 10 min at 12000 rpm to remove particulate matter and the supernatant was collected. The analysis process was conducted as detailed in Section 2.5.

3. Results and discussion

3.1. Characterization of Fe_3O_4@PPY

The morphology and microstructure of Fe_3O_4@PPY was characterized by TEM images (Fig. 1A). It is evident that Fe_3O_4 nanoparticles with a relatively uniform size and granular structure are intercalated into PPY polymer. The stability of Fe_3O_4@PPY was discussed in Section 1 of supplementary materials.

In the FT-IR spectra of Fe_3O_4@PPY (Fig. 1B), the peaks at 1545, 1456 cm^{-1} (the fundamental stretching vibration of pyrrole rings) and 1297 cm^{-1} (C—N stretching vibrations) could be examined, [22] and the other peaks at 1190, 1040, 920 and 789 cm^{-1} were due to in-plane and out-of-plane C—H and N—H bending vibrations [23]. All these peaks indicated the presence of PPY in Fe_3O_4@PPY. The shifting and merging of the peak from 570 cm^{-1} (reported in bulk Fe_3O_4) to 610 cm^{-1} corresponding to Fe-O stretching vibration confirmed the presence of Fe_3O_4 as well as the strong combination of Fe_3O_4 with PPY, rather than blending of the two components [24].