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A universal label-free fluorescent aptasensor based on Ru complex and quantum dots for adenosine, dopamine and 17β-estradiol detection

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ABSTRACT

Based on specific aptamer binding properties, a strategy for adenosine, dopamine and 17β-estradiol detection was realised by employing Ru complex and quantum dots (QDs) as fluorescence probes. Ru complex, which could quench the fluorescence of QDs, preferred to bind with aptamer DNA and resulted in the fluorescence rise of QDs. When the aptamer DNA was incubated with the target first, it could not bind with Ru complex and the fluorescence of QDs was quenched. Under the optimal condition, the fluorescence intensity was linearly proportional to the concentration of adenosine, dopamine and 17β-estradiol with a limit of detection (LOD) of 101 nM, 19 nM and 37 nM, respectively. The experiments in fetal bovine serum were also carried out with good results. This universal method was rapid, label-free, low-cost, easy-operating and highly repeatable for the detection of adenosine, dopamine and 17β-estradiol. Qualitative detection by naked eyes was also available without complex instruments. It could also be extended to detect various analytes, such as metal ions, proteins and small molecules by using appropriate aptamers.

1. Introduction

Human body is such a precise and ordered machine regulated by numbers of small molecules. Among all of them, adenosine (ADE), dopamine (DA) and 17β-estradiol (E2) play very important roles in the regulation processes and have attracted the investigators' attention for decades. ADE is the component of many biological cofactors and an endogenous modulator with potent vasodilator and antiarrhytmic activities which has received much attention due to its crucial signaling function in both the peripheral and the central nervous system (Spychala 2000; Yan et al., 2009). It also plays a fundamental role in many biological processes such as energy generation and protein metabolism. A number of investigations have been made to examine adenosine as possible biomarker for cancer (Yang et al., 2002). DA is an important neurotransmitter that plays many crucial roles in the cardiovascular, nervous, and renal systems and regulates various physiological activities (Li et al., 2013). Mammals' brains send out signals, through DA, to increase the breathing rate and blood circulation when they do not have enough oxygen (Urena et al., 1994). In the basal ganglia of the brain, DA is a neurotransmitter that plays a vital role in Parkinson's disease, which is a notorious nervous dysfunction associated with vibrating limbs during the early stages and dementia in the advanced stages (Kim et al., 2002). E2, as the most potent and active estrogen, can affect reproduction and the maintenance of sex characteristics. It can be absorbed from external sources, interfere with normal physiological processes, and create many deleterious effects (Yildirim et al., 2012). The quantification of estradiol levels in serum and urine is important in various clinical evaluations including investigations of fertility treatments, post-menopausal status, hyperandrogenism, and breast cancer (Kuramitz et al., 2003). Thus, developing simple, low-cost, high selective and sensitive sensors for rapid detection of ADE, DA and E2 is of great significance.

Many sensors for ADE, DA and E2 have been developed for decades. Conventional analytical methods, such as radioimmunoassay (RIA) (Siragy and Linden 1996), high-performance liquid chromatography (HPLC) (Ma et al., 2014) and capillary electrophoresis (CE) (Tzeng et al., 2006), have been successfully used to detect ADE. But these techniques/methods normally require time-consuming sample pretreatment processes, expensive instrumentation and well-trained technicians, all of which limit their application. With the development of biochemistry, some sensors with the advantages of portable, low-cost and rapid for ADE detection have been proposed involving various signal-transduction approaches such as fluorescence (Fu et al., 2013; Xiang et al., 2009), electrochemistry (Shandost-fard et al., 2014), electrochemiluminescence (ECL) (Zhu et al., 2011), colorimetry (Liu et al., 2011), and surface-enhanced Raman scattering (SERS)

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(Chen et al., 2008). But the flexibility and selectivity for ADE detection remain to be improved.

The determination of DA has been reported by using electrophoresis (Huang and Lin, 2005), chromatography (Uutela et al., 2009), HPLC (Muzzi et al., 2008), flow injection chemiluminescence (Nalewajko et al., 2004) and spectrophotometry (Carrera et al., 2007). However, all these methods generally require time-consuming sample preparation and expensive instrumental equipment, and the sensitivity and selectivity are not satisfied enough.

By the way, the conventional methods for E2 based on HPLC (Mahmoud et al., 2011) or GC/MS (Janssens et al., 2013) are accurate and sensitive, but the analytical procedures are expensive and time-consuming. Considering these limitations, some biosensors based on estrogen receptor and antibodies have been developed (Ko and Chang, 2014; Liu et al., 2009). However, the disadvantages of complicated, lacking of selectivity and narrow-application remain to be solved.

Considering of the advantages of small size, easy synthesis and modification, and strong binding affinity, aptamers offer a new alternative for small molecules detection and have been broadly used in the detection of drugs, cancer cells and a variety of proteins (Liu et al., 2009). The aptamers for ADE (Ko and Chang, 2014), DA (Zhou et al., 2014) and E2 (Long et al., 2014), have already been found and studied for many years as well.

Furthermore, quantum dots (QDs) have been extensively studied and utilized in biosensor designing for two decades. QDs have unique optical properties, such as broad excitation spectra, narrow, symmetric, and tunable emission spectra (Michalet et al., 2005). Compared with conventional organic fluorescent dyes, QDs exhibit remarkable advantages, including high fluorescence quantum yields, excellent photostability, and good water solubility, therefore they are more proper for multiplex detection (Smith and Nie, 2004). In recent years, several research groups have utilized luminescent quantum dots as donors in fluorescence resonance energy transfer (FRET) measurements (Díaz et al., 2012; Dennis et al., 2012; Shi et al., 2006; Wang et al., 2011). FRET between the QDs and the acceptors QSY9 (Medintz et al., 2003), rhodamine red (Medintz et al., 2004), Cy3 (Peng et al., 2007) and gold nanoparticles (Oh et al., 2005) has been reported proverbially. Polypyridine ruthenium complex, a kind of widely used DNA molecular "light switch" (Shi et al., 2010), can also work as FRET reagent for QDs (Amelia et al., 2011; Wu et al., 2011). Due to the excellent aptamer DNA binding property, Ru complex has been widely applied in biosensor designing (Xiang et al., 2012; Zhang et al., 2014b; Zhao et al., 2009).

Herein, we propose one system for ADE, DA and E2 detection. To achieve high selectivity and sensitivity, we take advantage of the specificity of aptamers and the excellent fluorescence property of CdTe QDs. The complex $Ru(bpy)_2dppz^{2+}$ (bpy=2,2′-bipyridine; dppz=dipyrido[3,2-a:2′,3′-c] phenazine) is utilized as both the fluorescence quencher to QDs and the receptor to aptamers. The fluorescence of QDs, quenched by Ru complex, can be restored by aptamer DNA. When the aptamer DNA is incubated with the target first, it can not bind with Ru complex and the fluorescence is quenched again. The fluorescence intensity is linearly proportional to the concentration of the target in proper range. In the earlier work of our group, we have realized the detection of thrombin by using this method (Sun et al., 2012). Now we enrich our work by utilizing this system to detect ADE, DA and E2, respectively. To the best of our knowledge, this method is one of the simplest sensors for ADE, DA and E2, and it has never been reported to detect these three chemicals by one universal system, which makes it of remarkable significance.

2. Experimental section

2.1. Materials and reagents

$[Ru(bpy)_2dppz]^{2+}$ was prepared and characterized according to the literature (Sun et al., 2012). The following chemicals: thioglycolic acid (TGA), $CdCl_2$, tellurium powder, $NaBH_4$, ADE, guanosine (G), thymine (T), cytidine (C), uridine (U), DA, ascorbic acid (AA), uric acid (UA), 3,4-dihydroxyphenylacetic acid (DOPAC), vanillic acid (VA), E2, progesterone (P4), testosterone (TT), bis(4-hydroxyphenyl) methane (BPF), bisphenol A (BPA), HCl, and NaOH were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China) and Aladdin Ltd. (Shanghai, China). Fetal bovine serum (FBS) was obtained from Sigma-Aldrich, in which the concentration of hemoglobin was about 20 mg/dL. The oligonucleotides used in this study were synthesized by Sangon Biotech Co. Ltd. (Shanghai, China):

DNA1: 5′-ACCTGGGGGAGTATTGCGGAGGAAGGT-3′;

DNA2: 5′-GTCTCTGTGTGCGCCAGAGAACACTGGGGCAGA-TATGGGC CAGCAC AGAATGAGGCCC-3′;

DNA3: 5′-AAGGGATGCCGTTTGGGCCCAAGTTCGGCATAGTG-3′.

The reaction solution for ADE and DA detection, named buffer-1, contained 10 mM Tris aminomethane with a pH of 7.4 regulated by HCl, and the solution for E2 detection, named buffer-2, contained 10 mM Tris aminomethane and 5% ethanol with a pH of 7.4 regulated by HCl. ADE, G, T, C, U, DA, AA, UA, DOPAC and VA were dissolved in buffer-1. E2, P4, TT, BPF and BPA were dissolved in buffer-2. The fresh stock solution of other chemicals was prepared in the triply distilled water.

2.2. Instruments

Fluorescence spectra with excitation at 365 nm were recorded at room temperature with an F-7000 fluorescence spectrophotometer (Hitachi Ltd., Japan) in a 1.0 cm path length quartz cuvette. The slit widths for both excitation and emission were fixed at 10 nm. UV–vis spectra were recorded at room temperature with a Lambda Bio 40 UV/Vis Spectrophotometer (Perkin-Elmer, USA) in a 1.0 cm path length quartz cuvette. High-resolution transmission electron microscopy (HRTEM) was performed on a JEM-2100 (JEOL, Japan) electron microscope operating at 200 kV. Photographs were taken under a 365 nm UV lamp with a WB351F digital camera (Samsung, South Korea).

2.3. Synthesis of TGA-capped CdTe quantum dots

The synthesis of CdTe QDs was performed according to the reference with some modification (Peng et al., 2007). First, NaHTe was prepared by adding 40 mg $NaBH_4$ to a flask containing 46 mg tellurium powder and 2 mL Milli-Q water under nitrogen atmosphere. The reaction was kept on for several hours until all tellurium powder was dissolved. 0.092 g (0.5 mmol) of $CdCl_2$ and 0.092 g (1 mmol) of thioglycolic acid were dissolved in 100 mL Milli-Q water, followed by adjusting pH to 8.2 by addition of 1 M NaOH solution. The mixture was deaerated by N_2 bubbling for 30 min. Then NaHTe solution (0.062 mmol) was quickly injected into the mixture under vigorous stirring, followed by refluxing the mixture for 2 h under open-air conditions. Then the as-prepared CdTe QDs were characterized by high resolution transmission electron microscopy (HRTEM), absorption and fluorescence spectroscopies, respectively.

2.4. Assays for ADE, DA and E2

In a typical ADE assay, sample solutions with different concentrations of ADE were first mixed with 1 μM DNA1 for 1 h at