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A sensitive quantum dots-based "OFF-ON" fluorescent sensor for ruthenium anticancer drugs and ctDNA

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ABSTRACT

In this contribution, a simple and sensitive fluorescent sensor for the determination of both the three ruthenium anticancer drugs (1 to 3) and calf thymus DNA (ctDNA) was established based on the CdTe quantum dots (QDs) fluorescence "OFF-ON" mode. Under the experimental conditions, the fluorescence of CdTe QDs can be effectively quenched by ruthenium anticancer drugs because of the surface binding of these drugs on CdTe QDs and the subsequent photoinduced electron transfer (PET) process from CdTe QDs to ruthenium anticancer drugs, which render the system into fluorescence "OFF" status. The system can then be "ON" after the addition of ctDNA which brought the restoration of CdTe QDs fluorescence intensity, since ruthenium anticancer drugs broke away from the surface of CdTe QDs and inserted into double helix structure of ctDNA. The fluorescence quenching effect of the CdTe QDs-ruthenium anticancer drugs systems was mainly concentration dependent, which could be used to detect three ruthenium anticancer drugs. The limits of detection were 5.5×10^{-8} M for ruthenium anticancer drug 1, 7.0×10^{-8} M for ruthenium anticancer drug 2, and 7.9×10^{-8} M for ruthenium anticancer drug 3, respectively. The relative restored fluorescence intensity was directly proportional to the concentration of ctDNA in the range of 1.0×10^{-8} M $\sim$ 3.0×10^{-7} M, with a correlation coefficient (R) of 0.9983 and a limit of detection of 1.1×10^{-9} M. The relative standard deviation (RSD) for 1.5×10^{-7} M ctDNA was 1.5% (n = 5). There was almost no interference to some common chemical compounds, nucleotides, amino acids, and proteins. The proposed method was applied to the determination of ctDNA in three synthetic samples with satisfactory results. The possible reaction mechanism of CdTe QDs fluorescence "OFF-ON" was further investigated. This simple and sensitive approach possessed some potential applications in the investigation of interaction between drug molecules and DNA.

1. Introduction

In the past two decades, the water-soluble semiconductor quantum dots (QDs) have attracted widespread attention as novel fluorescence indicator in diverse research areas, such as analysis, sensing, and biodetection regions [1–5]. The confinement of the excited electron and the holes in QDs generates the specific optical and electronic properties that are dramatically different from those in bulk semiconductors [6]. Compared to traditional organic dyes and fluorescent proteins, QDs have some unique photophysical properties, for example, the broad/continuous excitation spectrum and narrow/symmetric emission spectrum, high photobleaching threshold, high emission quantum yield, and so on [7–9]. These advantages make QDs to be excellent probes for some chemical and biological assay [10–12]. Some metal ions, small molecules, and biomacromolecules could be detected by using QDs as fluorescent probe based on fluorescence quenching or fluorescence enhancement phenomenon [13–19]. Nie and coworkers have reported an ultrasensitive and selective detection of Cu^{2+} with the fluorescence enhancement of CdSe QDs [13]. Zhou et al. have developed a novel luminescence sensing system for Sudan dye detection, based on the fluorescence quenching of oleic acid-functionalized Mn-ZnS QDs [15]. He's group further used the fluorescence increment phenomenon of CdSe/ZnS QDs to detect L-cysteine [17]. Ding et al. have explored specific and selective detection approach for

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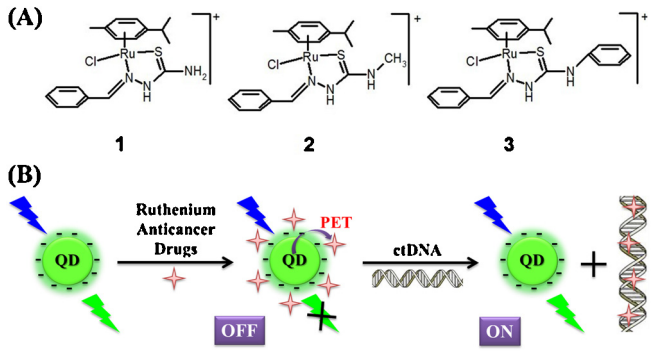

Fig. 1. A) The chemical structures of ruthenium anticancer drugs **1** to **3** after the detachment of the chloride ions. B) The schematic illustration of the principle of ruthenium anticancer drugs detection and ctDNA determination using the CdTe QDs-based "OFF-ON" fluorescent sensor.

bovine serum albumin (BSA) by fluorescence enhancement of Mn-modified CdTe QDs [19]. However, the fluorescence quenching and the fluorescence enhancement of QDs all belong to the unidirectional fluorescence variations of QDs which can be easily affected by some foreign substances. In order to expand the applications of QDs probe, new methods should be explored to increase the selectivity of QDs probe irrespective of any situation.

Fortunately, a novel and sensitive fluorescence "OFF-ON" sensor based on QDs had been explored recently [20–28]. In our previous work, through the specific and strong interactions between streptavidin and biotin or between single-stranded DNA and the complementary single-stranded DNA, the fluorescence of QDs were quenched efficiently by fluorescent dye due to the fluorescence resonance energy transfer (FRET). The fluorescence of QDs could be recovered partially after the addition of specific nuclease or targeted DNA because of the disturbance of FRET effects from QDs to fluorescent dye [20,21]. Since none of the common foreign substances could affect the FRET efficiency, this QDs-based "OFF-ON" fluorescent sensor ensured excellent selectivity in specific nuclease and targeted DNA detection. In addition, this fluorescence "OFF-ON" mode had attracted widespread interests and had realized simple and sensitive determination of different substances, such as Hg^{2+}, CN^{-}, mitoxantrone, DNA, nuclease, and viruses [22–28]. Furthermore, this novel mode could also be applied for the investigation of the interactions between some functional drugs and their targets [29,30]. Therefore, this new fluorescence "OFF-ON" sensor should be further studied to exploit their potential application in biochemical and biomedical detections.

As one kind of the organometallic compounds, ruthenium complexes have attracted great interests due to their specific antibacterial, antitumor, and anticancer activity [31–34]. The bioactivity effects of these ruthenium complexes are exerted by the inhibition of DNA or RNA replication and amplification after their intercalation into the double helix of DNA inside the cancer cells and then combination with the base pairs, which lead to the death of the cancer cells [35,36]. The investigation of the binding interaction of some bioactive ruthenium complex drugs to their target DNA has been an active area of research, which could make us better understand the mechanism of their antibacterial, antitumor, and anticancer activity, together with the directional design and the efficient synthesis of new functional drugs [37–39]. Until now, the binding interaction between different ruthenium complex drugs and their target DNA has already been investigated by many approaches [40–48]. However, to our knowledge, the application of QDs as the fluorescent sensor to test the ruthenium anticancer drugs-DNA interaction has rarely been reported till now.

Herein, we establish a simple and sensitive QDs-based "OFF-ON" fluorescent sensor by utilizing ruthenium anticancer drug as both the quencher to QDs and the intercalating agent to calf thymus DNA (ctDNA) (Fig. 1). It is well known that the possible quenching mechanism of ruthenium complex to QDs is mainly photoinduced electron transfer (PET) process [49,50]. As shown in Fig. 1, based on the electrostatic interactions, the negatively-charged CdTe QDs could be combined with the positively-charged ruthenium anticancer drugs after the detachment of the chloride ions. Because of the surface combination of ruthenium anticancer drugs on CdTe QDs, PET could occur easily between CdTe QDs and ruthenium anticancer drugs. Meanwhile, ruthenium (II), which is the central ion of the ruthenium anticancer drugs, has partially filled d-orbitals and can be used as electron acceptor. The ultrafast PET from CdTe QDs to ruthenium anticancer drugs prevented the normal recombination of electron and hole in CdTe QDs, which resulted in the fluorescence "OFF" status of CdTe QDs. After the addition of ctDNA, the positively-charged ruthenium anticancer drugs could combine with the negatively-charged ctDNA through electrostatic forces and insert into the double helix structure of ctDNA [35,37,38]. Since the mutual repulsion between both the negatively-charged ctDNA and the negatively-charged CdTe QDs existed, the ctDNA-ruthenium anticancer drug complexes could break away from the surface of CdTe QDs, and this interrupted the electron transfer from CdTe QDs to ruthenium anticancer drugs and rendered the fluorescence "ON" status of CdTe QDs. This QDs-based "OFF-ON" fluorescent sensor could be used to detect both ruthenium anticancer drugs and ctDNA with the properties of simplicity, rapidity, and sensitivity.

2. Experimental

2.1. Reagents

Tellurium (powder, 200 meshes, 99.8%), sodium borohydride ($NaBH_4$, 99.8%), $CdCl_2{\bullet}H_2O$ (99.99%), and *N*-acetyl-*L*-cysteine (NAC, ≥ 99%) were purchased from Sigma (St. Louis, MO, USA). ctDNA, nucleotides, and amino acids were obtained from Sinopharm Chemical Reagent Factory (Shanghai, China). Three ruthenium anticancer drugs (**1** to **3**) were synthesized according to the reference reported previously [51]. BSA, papain, lysozyme, and pepsin were purchased from Beijing Huamei Bioscience Technology (Beijing, China). All other reagents were of analytical-reagent grade and used as received without further purification. Ultrapure water with a resistivity of 18.2 MΩ cm was produced by passing through a RiOs 8 unit followed by a Millipore-Q Academic purification set (Millipore, Bedford, MA, USA) and used throughout the experiments.

2.2. Apparatus

The absorption spectra were measured on a TU-1901 UV-vis spectrophotometer (Beijing Purkinje General Instrument Co., Ltd., Beijing, China). All fluorescence spectra and intensities were recorded with a Perkin-Elmer Model LS-55 luminescence spectrometer (PerkinElmer, Waltham, MA, USA) equipped with a 20KW xenon discharge lamp as light source. Quartz cells (1 cm path-length) were used for all measurements. The time-resolved fluorescence decay traces were recorded with a Fluorolog-3 system (Horiba Jobin Yvon, France) by using an excitation wavelength of 374 nm. All pH measurements were made with a basic pH meter PB-10 (Sartorius Scientific Instruments Co., Ltd., Beijing, China).

2.3. Preparations of CdTe QDs

The CdTe QDs were synthesized according to the method described previously [52,53]. In a typical synthesis, 0.2 mmol tellurium powder and 1.0 mmol $NaBH_4$ were put into a 25 mL two-necked flask equipped with a constant pressure funnel which contained 5.0 mL ultrapure water. Air in this system was pumped