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Label-free sensing of thrombin based on quantum dots and thrombin binding aptamer

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ABSTRACT

A facile and sensitive label-free approach for detection of thrombin based on CdTe quantum dots (QDs) and thrombin binding aptamer (TBA) is presented. The crude QDs can be "activated" with fluorescence enhancement by adding extra Cd^{2+} to the solution in basic medium. As a result, the positively charged Cd^{2+}-activating CdTe QDs could interact with the negatively charged TBA, leading to fluorescence quenching. When thrombin was added, TBA was induced to form a G-quadruplex structure and combined specifically with its target, releasing the QDs with a recovery of the fluorescence intensity. The sensing approach is based on the strongly specific interactions between TBA and thrombin over the electrostatic interactions between TBA and positively charged QDs. Based on the fluorescence enhancement of QDs, selective detection of thrombin was successfully achieved. A linear response for thrombin was observed in the range from 1.4 nM to 21 nM with a detection limit of 0.70 nM.

1. Introduction

Quantum dots (QDs) are semiconductor nanoparticles or alloys between 1 nm and 10 nm in size. Since its first appearance in 1983 [1], semiconductor QDs have been received broad research interest. Especially from 1998, when Chan and Nie [2] along with Bruchez et al. [3] prepared water soluble CdSe/ZnS core/shell QDs and utilized them for cellular imaging, researches focusing on biological applications of QDs have been paid increasing attention. Compared with conventional organic fluorophores, QDs display favorable photophysical properties, including size-controlled fluorescence, broad absorption and excitation spectra, narrow and symmetric fluorescence emission band, and high stability against photobleaching [4]. Thus, QDs have been widely utilized as excellent optical labels for biological imaging, sensing, and diagnostics [5–8].

The nature of the quantum dots surface is critical for photoluminescence properties. The fluorescence efficiency of the prepared QDs could be significantly reduced by localized surface trap states, especially for QDs synthesized in aqueous media [9]. In order to reduce non-radiative recombination, and to increase the photoluminescence efficiency, several methods could be used. For example, (1) Overcoating the QDs core with one or two semiconductor quantum shells with a larger band-gap [10]. (2) Surface passivation through treatments with metal ions like Cd^{2+} or Zn^{2+} [9]. (3) Photoactivation through exposure of QDs to UV or visible light [11].

Thrombin is a specific serine protease that plays a critical role in hemostasis. It converts soluble fibrinogen into insoluble strands of fibrin, as well as catalyzes many other coagulation-related reactions. Thrombin also has great importance in molecular biology, for instance, the recognition of tumor growth, metastasis, and angiogenesis [12]. Due to its biological significances, a lot of efforts have been done to develop simple and sensitive approach for detection of thrombin [13–19]. Among these, aptamer based methods are the mostly used strategies because of the excellent specificity of thrombin binding aptamer toward thrombin [20,21]. The 15-mer thrombin binding aptamer, with the sequence of 5′-GGTTGGTGTGGTTGG-3′, was selected from a randomized oligonucleotide library as a high affinity ligand for thrombin [22]. It could form a G-quadruplex structure consisting of two guanine quartets upon interactions with thrombin [23].

Different methodologies have been used to transduce the aptamer-target binding events to detectable physical signals, including colorimetry [24], electrochemistry [25,26], fluorescence spectra [27–29], capillary electrophoresis [30,31] and so on. However, in most methods, the aptamers were usually either modified with fluorophores or fixed onto a certain surface, like electrodes, through covalent coupling reactions, using the specific interactions between streptavidin and biotin, NHS and EDC, SH and metal elements like Au or Cd^{2+}, etc. Such covalent modifications are usually labor-intensive and time-consuming, and also it would lower the binding abilities of aptamers toward targets

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[32,33]. A large number of the methods reported were based on turn-off signals, which would lower the sensitivity of the probes due to the relatively high background with the decrease of intensity [34]. Here, we designed a label-free turn-on type fluorescent method to detect thrombin via monitoring the fluorescence changes of QDs induced by thrombin upon binding with its aptamer. This method for thrombin sensing has advantages of easy operation due to the elimination of complicated covalent modifications. The absence of modification could ensure the free conformational change of the aptamer when interacting with thrombin, thus leading to high binding affinity and sensitive detection. The results showed that this method was highly sensitive and selective in thrombin sensing, even in a complex biological matrix like serum.

2. Experimental section

2.1. Materials and reagents

Tellurium powder (approximately 325 mesh, 99.99%), 3-mercaptopropionic acid (3-MPA, 99%), and quinine hemisulfate monohydrate (99%) were from Alfa-Aesar. Cadmium chloride hemipentahydrate ($CdCl_2 \cdot 2.5\ H_2O$) and sodium borohydride (96%) were purchased from Sinopharm Chemical Reagent Company. Thrombin was purchased from Sigma. 15-mer thrombin binding aptamer (TBA) with the sequence of 5′-GGTTGGTGTGGTTGG-3′ was synthesized by Beijing Dingguo Biotechnology Company and was stored in 10 mM Tris–HCl buffer (pH=7.4) containing 1 mM ethylenediaminetetraacetic acid. Bovine serum albumin (BSA), lysozyme and immunoglobin G (IgG) were also purchased from Beijing Dingguo Biotechnology Company. Tris (hydroxymethyl) aminomethane (Tris) was purchased from Novon, Incorporation. Sulfuric acid, hydrochloric acid, sodium hydroxide, ethanol, and 2-propanol of analytical grade were all from Beijing Beihua Fine Chemical Company. The fresh human blood samples from healthy subjects were supplied by the affiliated hospital of Beijing Normal University. After careful settlement for 2 h, the supernatant was extracted as serum and then centrifuged thrice (2500 rpm, 10 min once). The serum samples were diluted five times prior to detection. The ultrapure water used throughout the experiments has a resistivity of more than 18 MΩ cm.

2.2. Apparatus

Fluorescence spectra were recorded using a Hitachi F-4600 fluorescence spectrometer, and UV–visible absorption spectra were recorded on a TU-1901 diode-array spectrophotometer. High-resolution transmission electron microscopy (HRTEM) was performed on a JEM-2010 (JEOL, Japan) electron microscope operating at 200 kV. The pH values of solution were determined by a REX PHS-3C pH meter. A stirrer with temperature sensor and a thermometer was used to control the temperature precisely.

2.3. Preparation of CdTe QDs

The CdTe QDs were prepared via a green synthetic route according to the reported method [35] with minor modification. Briefly, under a N_2 atmosphere, absolute ethanol (3 mL) was added to tellurium (6.8 mg) and excess sodium borohydride (18 mg) under magnetic stirring, which is kept at 70 °C for 1.5 h. The resulting pink ethanol solution of NaHTe was reacted with H_2SO_4 (50 mM, 5 mL) to produce H_2Te gas. Under stirring, H_2Te gas was introduced into the oxygen-free $CdCl_2$ (3.03 mM, 50 mL) aqueous solution containing 3-mercaptopropionic acid (MPA) (45 μL) stabilizer at pH 9.0 by a N_2 flow, resulting in the CdTe precursor solution. The initial molar ratio of Cd/Te/MPA was approximately 3:1:10. Then the above obtained CdTe precursor solution was refluxed at 100 °C for 5 h under open air conditions with a condenser attached. Calculated by the first absorption maximum according to a reported method [36], the CdTe QDs obtained were 3.0 nm in size. Isolation of 3-MPA capped CdTe QDs from their crude solution was achieved by precipitation with an equal volume of 2-propanol, and the sediment was collected by centrifugation at 6000 rpm for 5 min. This procedure was repeated for three times before the sediment was redissolved in double distilled water and then stored at 4 °C for the subsequent experiments.

Then the as-prepared CdTe QDs were characterized by high resolution transmission electron microscopy (HRTEM), absorption and fluorescence spectroscopies, respectively. The photoluminescence quantum yield (QY) of CdTe QDs was estimated using quinine sulfate (QY=54.6%) [37] as reference.

2.4. Thrombin detection

The originally prepared QDs were first activated as following: The solution of the QDs was adjusted to pH 10.5 by addition of 0.5 M NaOH; then, certain amount of extra $Cd(ClO_4)_2$ solution was added. The reaction was monitored by determining the fluorescence intensity excited at 370 nm. The addition of Cd^{2+} stopped when the fluorescence intensity no longer showed obvious increase.

After QDs activation was performed under optimum conditions, TBA (4.35 μM, final concentration) was added into the Cd^{2+}-activating CdTe QDs solution. And then, the emission spectra were recorded after 30 min of equilibration time. Then, for thrombin detection, different concentrations of thrombin were added into the above Cd^{2+}-activating CdTe QDs–aptamer solution with incubation at 37 °C for 30 min, and then the fluorescence intensity was monitored.

Each measurement was conducted three times to estimate the error value of one data point.

3. Results and discussion

3.1. Characterization of CdTe QDs

The photo-physical properties of the as-prepared QDs were characterized using UV–visible absorption spectra and fluorescence emission spectra, as illustrated in Fig. 1. The absorption maximum of the first electronic transition was at 520 nm. The fluorescence spectra of the CdTe QDs showed an emission maximum at 563 nm upon excitation at 370 nm. The well-resolved sharp first excitonic peak in the absorption spectra and narrow FWHM (full width at half maximum) in the fluorescence emission spectra indicated a narrow size distribution of the as-prepared CdTe QDs [38]. The photoluminescence quantum yield (QY) of the CdTe QDs was determined to be 19%.

High resolution transmission electron micrograph (HRTEM) was employed to characterize the morphology of the as-synthesized CdTe QDs, as was shown in Fig. 2. The CdTe nanocrystals appeared as roughly spherical particles and possessed a relatively good monodispersity and crystallinity. The average diameter of the CdTe nanoparticles was 3.2 nm ± 0.2 nm.

3.2. Efficient activation effect of Cd^{2+} on CdTe QDs

The activation of QDs is typically attributed to some forms of surface passivation. In this paper, surface passivation via treatment of QDs with metal ions was applied due to its high efficiency and convenience. Upon addition of Cd^{2+}, surface defect states were passivated, and the CdTe QDs were thus activated