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A ratiometric magnesium sensor using DNAzyme-templated CdTe quantum dots and Cy5

Jidong Wang[a,*], Xiaoyu Wang[a], Hengshan Tang[a], Shengquan He[a], Zehua Gao[a], Ruixu Niu[a], Yue Zheng[b], Shumin Han[a,c,*]

[a] Hebei Key Laboratory of Applied Chemistry, School of Environmental and Chemical Engineering, Yanshan University, Qinhuangdao 066004, China
[b] The First Hospital in Qinhuangdao Affiliated to Hebei Medical University, Qinhuangdao 066004, China
[c] State Key Laboratory of Metastable Materials Science and Technology, Yanshan University, Qinhuangdao 066004, China

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ABSTRACT

DNAzyme-templated CdTe quantum dots (DNAzyme-CdTe QDs) were synthesized through a facile one- step route and the obtained QDs were used to fabricate a novel ratiometric magnesium sensor. Crosslinking and surface modification process of QDs were not the necessary step in this synthesis. By catalyzing the DNAzyme sensing system, composed of DNAzyme- CdTe QDs and Cy5 labeled substrate strand, Mg^{2+} was detected. The fluorescent intensity ratio between CdTe QDs and Cy5 showed a good linear relationship with Mg^{2+}concentration. The sensor displayed a wide range of linear response (0–20 nM), low detection limit (0.3 nM, (S/N = 3)), good reproducibility, stability and selectivity.

1. Introduction

DNAzymes as an artificial deoxyribozyme, due to their highly specific and sensitive for metal ions, have conjugated with various signaling transduction strategies to detect metal ions [1–5]. Semiconductor quantum dots (QDs), for their excellent optical characters and outstanding signal transduction capacity, have attracted great interest in the biosensing community [6–8]. These unique characters make DNAzyme-QDs system as a promising platform using for detecting metal ions in environmental samples and imaging in living cells [9–12]. However, so far, during the synthesis process of DNAzyme-QDs, Crosslinking and surface modification of QDs by specific cross-linking agent, such as EDC/NHS et al., is the essential and prior step.

Magnesium ion, as an alkaline earth metal ion, plays an essential role in biological activities. However, due to lack of specific recognizing probe, the efficient methods for detecting Mg^{2+} are limited. Herein, we reported a ratiometric sensing synthesis for detecting Mg^{2+} using Mg^{2+}-dependent DNAzyme- CdTe QDs and Cy5. A facile one-step approach was proposed to synthesize DNAzyme- CdTe QDs, in which the process of crosslinking and surface modification with DNAzyme was not the necessary step. Two different domains were designed in the modified DNAzyme strand, including phosphorothioates(*ps*) and phosphates (*po*) backbone which served as synthetic agent and corresponding DNAzyme strand, respectively. After DNAzyme strand had hybridized with Cy5- labeled DNAzyme substrate strand (S-DNA), the fluorescence of CdTe QDs was quenched by Cy5 due to fluorescence resonance energy transfer(FRET) between QDs and Cy5. When Mg^{2+} was added into the sensing system, the DNAzyme was catalyzed and substrate strand was cleaved. The small DNA segment with Cy5 fled into solution so that the fluorescence of CdTe QDs recovered. The ratio of signal intensity between QDs and Cy5 showed a good linear relationship with Mg^{2+} concentration. Therefore, a novel biosensor for detecting Mg^{2+} was obtained (Scheme 1).

2. Experimental

2.1. Materials and reagents

Sodium borohydride ($NaBH_4$, 98%), sodium hydroxide (NaOH, 96%), anhydrous ethanol (99.7%), Cadmium chloride ($CdCl_2$, 99%), anhydrous magnesium chloride ($MgCl_2$, 99%), 3-mercaptopropionic acid (MPA, 98%), Tris-HCl buffer solution, sodium borohydride ($NaBH_4$, 95%), sodium tellurite ($NaTeO_3$, 99.0%) were obtained from Alfa Aesar. DNAzyme: G*G*G*G*G AAAAATTTTGTCAGCGAT CCGGA ACGGC ACCCATGTGAGAGAA (* indicates the phosphorothioate linkage) and S-DNA: T TCT CTC T rA G GAC AAA A-Cy5 were synthesized by Shanghai Sangon Biotechnology Co. Ltd.

* Corresponding authors at: College of Environmental and Chemical Engineering, Yanshan University, Qinhuangdao, 066004, China.
E-mail addresses: wangjidong@ysu.edu.cn (J. Wang), 793451503@qq.com (X. Wang), 1262754703@qq.com (H. Tang), 2252089989@qq.com (S. He), 641830880@qq.com (Z. Gao), 1965714984@qq.com (R. Niu), yuezheng1965@sina.com (Y. Zheng), hanshm@ysu.edu.cn (S. Han).

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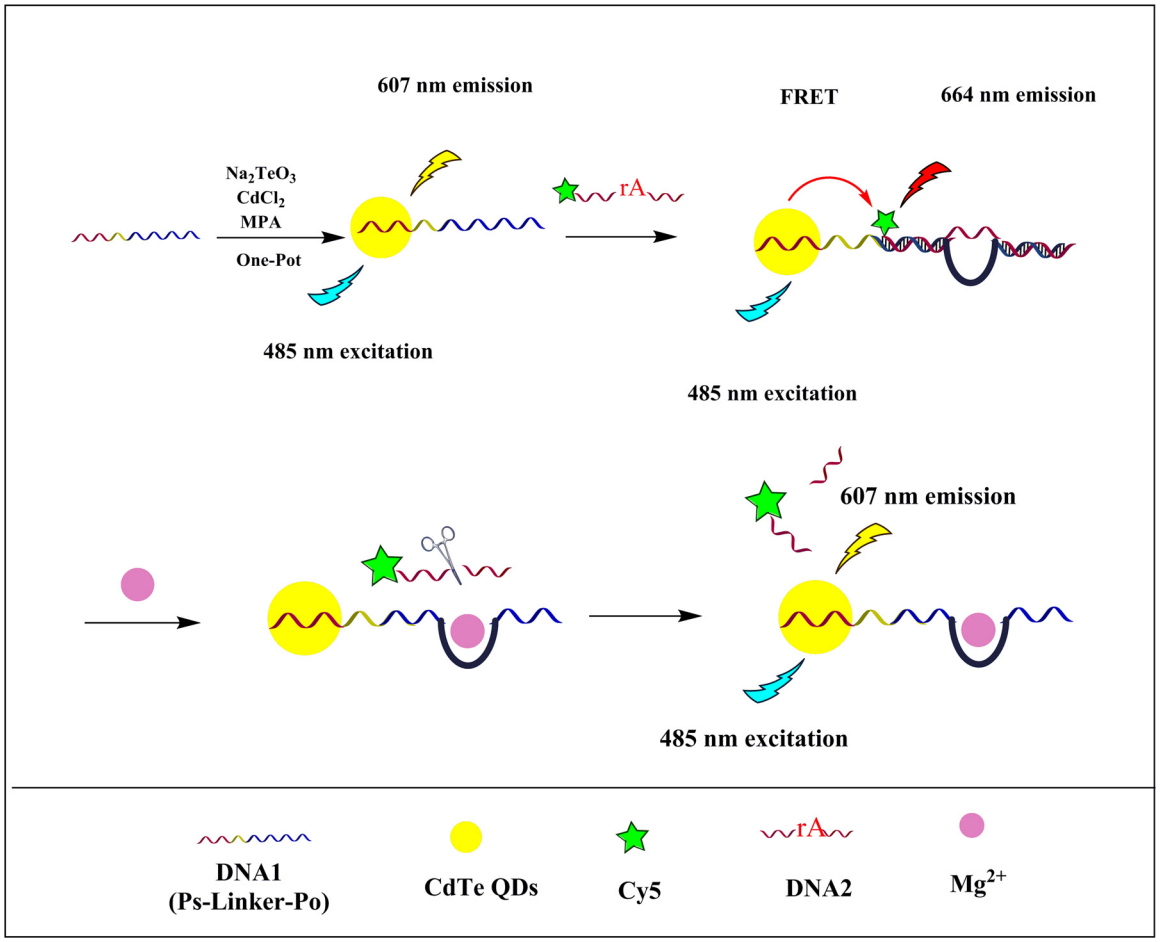

Scheme 1. Schematic illustration for one-pot synthesized DNAzyme-CdTe QDs for highly sensitive detection of Mg^{2+}.

2.2. Apparatus

UV–vis absorption spectroscopy and fluorescence spectrum measurements were recorded on a UV-2550 (Shimadzu, Kyoto, Japan) and F-7000 (Hitachi, Tokyo, Japan). Transmission electron microscopic (TEM) was obtained by a HT7700 transmission electron microscope (Hitachi, Tokyo, Japan). FTIR spectral analysis was recorded by a Nicolet iS10 spectrometer (Thermo Fisher Scientific Co., Madison, WI).

2.3. Preparation of DNAzyme – templated CdTe QDs

$CdCl_2$ (0.0183 g), MPA (0.0106 g) and deionized water (50 mL) were loaded in flask together. The mixture solution agitated and pH value was adjusted to 9.0. Then, Na_2TeO_3 (0.0056 g) and $NaBH_4$ (0.0189 g) were added to provide a Cd^{2+} to MPA to TeO_3^{2-} molar ratio of 1:1:0.25 solution. 10 μL DNAzyme (10 μM) was added in 1 mL above mixture solution and heated to 90 °C. The crude DNAzyme-CdTe QDs samples were washed by deionized water and alcohol three times.

2.4. Establishment of the sensing system for Mg^{2+}

160 μL DNAzyme – CdTe QDs (about 50 μM) and 160 μL S-DNA-Cy5 with some concentration complementary strand were successively added into 0.5 mL calibrated test tube. Then the solution was diluted to 400 μL with Tris-HCl (pH 8.0, 0.1 mol/L) followed by the shaking and incubating for some time at 37 °C. Then, 20 μL Mg^{2+} (0, 1, 5, 10, 20, 40, 100, 500 nM) was added and shaked for 60 min. The PL intensity was investigated by fluorescence spectrum (λ_{ex} = 485 nm).

3. Results and discussion

3.1. Synthesis and characterization of the DNAzyme – CdTe QDs

The synthesis conditions of DNAzyme – CdTe QDs, including the reaction time, pH, the concentration of DNA and the number of *ps*, were discussed as shown in Fig. 1. The optimum parameters were chosen as follows: the reaction time was 2 h, pH value was 9.0, concentration of DNA1 was 0.03 nM and *ps* modified G number was five.

The distinct exciton absorption peak of DNAzyme – CdTe QDs appeared at 545 nm and a strong fluorescence emission peak was at 607 nm as Fig. 2A shown. The emission spectrum of CdTe QDs would share a broad overlapping with the absorbance of Cy5, because the absorption position peak of Cy5 was about 650 nm as shown in Fig. 2B. The CdTe QDs and Cy5 could be as the donor-acceptor pairs to promise an efficient FRET happened. According to the Peng's empirical formula [13], we could calculate that the size of CdTe QDs was about 3.1 nm. TEM analysis of as-synthesis QDs also indicated that these QDs had spherical shapes and the average size was about 3.1 nm as shown in Fig. 2C, which was consistent with the calculation of above empirical equation. FT-IR was investigated as shown in Fig. 2D. The functional groups belong to DNAzyme sequence appeared including the stretching vibrations of C═O (1560 cm^{-1}) and −OH (3436 cm^{-1}), the P—O stretches of the main chain (880 cm^{-1}), the $-NH_2$ feature (1620 cm^{-1}), the out of phase symmetrical stretches (1040 cm^{-1}), and the asymmetric stretching vibrations of the PO^{2-}(1380 cm^{-1}). The results confirmed that the DNAzyme strand as ligand agent had participated in the synthesis and had successfully coordinated on the surface of CdTe QDs.