Analytical Methods

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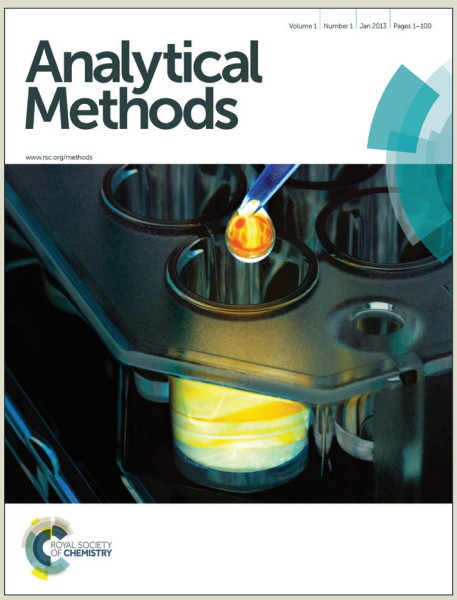

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PAPER

Aptamer-Functionalized CdTe:Zn^{2+} Quantum Dots for the Detection of Tomato Systemin

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As the advantages of the excellent optical properties and biocompatibility, DNA-functionalized quantum dots (QDs) have been widely applied in biosensing and bioimaging. Systemin, is an important class of plant peptide hormone firstly identified in plant. In this paper, we have synthesized aptamer-functionalized Zn^{2+} doped CdTe QDs through a facile one-pot hydrothermal route, and a fluorescent aptasensor based on graphene oxide (GO) is developed for the detection of tomato systemin (TomSys) with the recognition properties of aptamer. In the absence of TomSys, aptamer-functionalized QDs are adsorbed on the surface of GO and the fluorescence is efficiently quenched. While in the presence of TomSys, the specific binding of TomSys with its aptamer competitively releases aptamer-functionalized QDs from the GO surface, leading to the recovery of QDs fluorescence. The results demonstrate that the simple, rapid and cost-efficient biosensor possesses satisfactory sensitivity and selectivity for the detection of TomSys.

1. Introduction

In the past two decades, semiconductor quantum dots (QDs) have attracted widespread attentions in the diverse research areas, such as biosensing, biological imaging, immunoassay, and drug delivery because of their unique photophysical properties [1-3]. By conjugating protein, peptide, and DNA with QDs, a number of QDs-based nanoprobes have been employed in the bionanotechnology field. Among these applications, DNA-functionalized QDs have been widely applied in biosensing, bioimaging, and self-assembly [4-7]. Up to now, three common methods have been used to construct conjugated DNA-QDs, such as strepavidin-biotin [8], EDC/NHS conjugation [9], and ligand exchange [10,11]. But all of these methods still have several limitations, such as high cost, multistep synthesis and complicated functionalization. However, our group have previously developed a one-step hydrothermal route to synthesize DNA-functionalized Zn^{2+} doped CdTe QDs which have been successfully applied in the detection of hepatitis B virus surface-antigen gene [5], and active tumor-targeted imaging in vitro and in vivo [12]. It is worthy to note that, compared with CdTe QDs, the toxicity of DNA-functionalized CdTe:Zn^{2+} QDs is reduced considerably because of the high Zn doped ratio and the existence of DNA.

Recently, graphene oxide (GO) has attracted great interest in biological applications, owing to its unique characteristics such as good water solubility [13], versatile surface modification and superior fluorescence quenching ability [14]. Based on these properties, GO is widely used as universal highly efficient quencher to develop many biosensors. Therefore, GO-based sensors have been developed for the detection of DNA [15], proteins [16], enzyme activity [17], and other small molecules [18].

Systemins are a class of peptide hormones in plant that play a vital role in the regulation of plant defense, reproduction, growth, and development [19-23]. It has been found in the plant species of the solanaceae family, including potato, tomato, bell pepper, and black nightshade [24,25]. For example, in tomato plants, systemin is a systemic wound signal hormone in their leaves and activates the synthesis of proteinase inhibitors in their tissues to disrupt the herbivore and pathogen attacks as well as other mechanical woundings [26-29]. The sequence of tomato systemin (TomSys) is AVQSKPPSKRDPPKMQTD. Several methods have so far been applied in the isolation or analysis of TomSys, such as LC-UV, LC-MS, CE-LIF and CE-UV [24,30,31,32], while very limited works have been reported on its quantitative detection [31,32]. Shangguan's group [33] has reported the selection of a group of DNA aptamers that can specifically bind to TomSys, and designed an aptasensor to further confirm the obtained aptamer possesses strong affinity to TomSys. The screening of the aptamer sequence is of significance for chemical analysis and biological investigation of TomSys. However, the sensitivity of the aptasensor which is constructed based on the aptamer beacon is far from satisfactory. In this study, we propose a simple, specific and rapid strategy for the detection of TomSys based on GO. To

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the best of our knowledge, the analytical detection of the peptide hormone in plant is described for the first time by the one-step synthesis of DNA-functionalized QDs.

2. Materials and methods

2.1. Chemicals and apparatus

$CdCl_2 \cdot 2.5H_2O$, $ZnCl_2$, tellurium (reagent powder), and sodium borohydride ($NaBH_4$) were obtained from Sinopharm Chemical Reagent (China). N-acetylcysteine (NAC), sodium phosphate monobasic dihydrate ($NaH_2PO_4 \cdot 2H_2O$), sodium phosphate dibasic (Na_2HPO_4), potassium chloride (KCl), sodium chloride (NaCl) were commercially available from Sigma (USA). TomSys (98.33%, the sequence: AVQSKPPSKRDPPKMQTD), peptide (96.72%, the sequence: VVVT) were synthesized by ChinaPeptides Co., Ltd. (China). MS plant tissue culture was purchased from Shanghai haling Biotechnology Co. Ltd. (China). GO was purchased from Sinocarbon Materials Technology Co., Ltd. All chemicals used were of analytical grade or of the highest purity available and the solutions were prepared by a Milli-Q water system (Millipore Corp., USA) with resistivity of 18.2 M Ω cm. Modified aptamer, 5′-CGGGTTTCGGGGGGGTAGGGAGGAAAAA*G*G*G*G*G*G*G*G*G-3′ (* indicates the phosphorothioate linkage) was synthesized by Shanghai Sangon Biotechnology Co. Ltd. (China).

Fluorescence spectra were collected on a RF-5301 spectrofluorometer (Shimadzu, Japan). UV-vis absorption spectra were obtained using a UV-2550 spectrophotometer (Shimadzu, Japan). Fluorescence intensity decay curves were measured on QM/TM system (Photon Technology International). Transmission electron microscopy (TEM) and high-resolution (HR) TEM images were obtained with a JEOL Ltd. JEM 2100 electron microscope (Japan). All optical measurements were performed at room temperature under ambient conditions.

2.2. Preparation of aptamer-functionalized Zn^{2+} doped CdTe QDs

In a typical synthesis, $NaBH_4$ (20 mg) was reacted with Te powder (25 mg) in deionized water (1.0 mL) to produce NaHTe solution at 0 ° C for 5 h. The mixture of Zn^{2+} and Cd^{2+}-NAC solution was prepared by dissolving $CdCl_2 \cdot 2.5H_2O$, $ZnCl_2$, and NAC in deionized water. The pH of the solution was adjusted to 9.0 by dropwise addition of 1.0 M NaOH solution. Then, the fresh NaHTe solution (0.4 mL) was injected into a N_2-saturated mixture of Zn^{2+} and Cd^{2+}-NAC precursor solution. The typical molar ratio of Cd, Zn, Te, and NAC introduced was 1:2:0.2:3.6. Afterwards, DNA solution containing 2.5 μ M nucleotides was added to the mixture. Finally, the mixture was moved to a Teflon-lined stainless steel autoclave and heated to the desired growth temperature (200 ° C). The reaction solution was purified with a 30 K ultrafiltration tube by ultrafiltration using an Amicon Ultra-4 centrifugal filter device. The as-prepared products were stored at 4 ° C for further use.

2.3. Aptasensor based on GO for TomSys detection

Firstly, different concentrations of TomSys were added into the solution of aptamer-QDs (26 nM), and the mixtures were incubated for 15 min in sodium phosphate buffer (15 mM Na_2HPO_4/NaH_2PO_4, 140 mM NaCl, 4 mM KCl, pH 7.4). Then, GO (53.3 μ g/mL) was added to the mixtures and incubated for 5 min before measurement. Fluorescence emission spectra of the QDs were recorded under excitation at λ = 350 nm.

3.Results and discussion

3.1. The principle of detection

Figure 1 depicted the principle of the analytical process for the detection of TomSys. Aptamer-functionalized Zn^{2+} doped CdTe QDs were prepared with NAC ligand by a one-pot method [34,35]. As the coligand, the phosphorothiolate phosphate (ps-po) DNA [5,12,36] contained two domains, phosphorothioates which binded to QDs because of their high affinity to cadmium, and functional domain which interacted with biomolecular targets. In the absence of TomSys, aptamer-QDs were adsorbed on the surface of GO through a strong noncovalent binding by π - π stacking interactions [37] and the fluorescence was effectively quenched. While in the presence of TomSys, aptamer binded to TomSys and aptamer-functionalized QDs kept away from the surface of GO, which resulted in the obvious fluorescence emission of the reaction system. Therefore, fluorescent detection of TomSys could be easily realized by monitoring the fluorescence signal change.

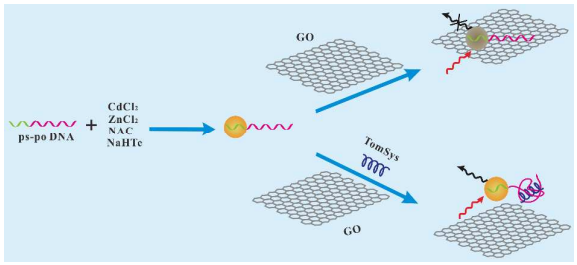

Figure 1. Schematic representation of the designed aptasensor for the detection of TomSys.

3.2. UV-Vis absorption and fluorescence spectra of aptamer-QDs and QDs

Figure 2 showed the UV-vis spectra and the fluorescence spectra of aptamer-QDs and QDs. The absorption of aptamer-QDs around λ = 260 nm was clearly due to the UV absorption of DNA, indicating that the aptamer was successfully attached to the QDs (Figure 2A). As shown in Figure 2B, aptamer-QDs and QDs showed the similar fluorescence spectra, and they both had narrow emission spectrum. It was indicated that the luminescence properties of the QDs weren't influenced by

the DNA. In addition, the maximum emission peak of aptamer-QDs was 586 nm.

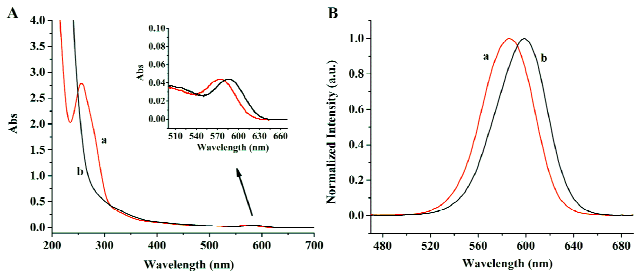

Figure 2. (A) UV–vis spectra of (a) aptamer-QDs and (b) QDs. (B) The normlized fluorescence emission spectra of (a) aptamer-QDs and (b) QDs.

3.3. Characterization of aptamer-QDs and QDs

The typical transmission electron microscopy (TEM) image of CdTe:Zn^{2+} QDs revealed that the QDs were well dispersible (Figure S1). Compared with the TEM image of QDs, the aptamer-QDs also showed excellent monodispersity as seen from Figure 3A. In addition, it revealed that aptamer-QDs were nearly spherical morphology and the particle sizes were about 3.5 nm (Figure 3B). The highly crystalline structure of the aptamer-QDs was exhibited clearly on the high resolution TEM (HRTEM) image (inset in Figure 3A). According to the fluorescence lifetime decay curves displayed in Figure 3C, both of the QDs and the aptamer-QDs exhibited long fluorescence decay times. The emission decay times of aptamer-QDs and QDs were 28.61 $\pm$ 3.67 ns and 26.02 $\pm$ 4.06 ns, respectively, indicating that the fluorescence decay time of aptamer-QDs was slightly longer than that of QDs.

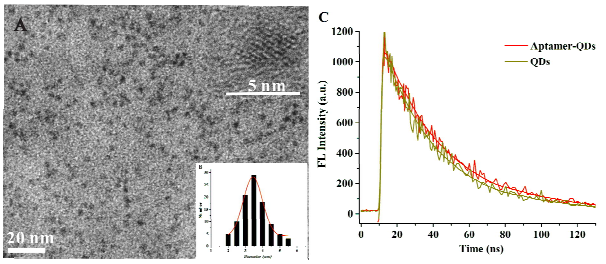

Figure 3. (A) TEM image of aptamer-QDs (inset: the HRTEM image). (B) Size distribution of aptamer-QDs. (C) Fluorescence lifetime decay curves of aptamer-QDs and QDs (λ_{ex} = 337 nm).

3.4. The feasibility of the strategy

To demonstrate the feasibility of this strategy, the fluorescence intensity of this system under different conditions was measured. As shown in Figure 4, the system containing only aptamer-QDs exhibited strong fluorescence emission (curve a). Upon the addition of GO, only a weak fluorescence emission could be observed due to the quenching caused by GO (curve b). However, in the presence of TomSys, a significant fluorescence signal was obtained in the addition of GO (curve c). These results indicated that the synthesized aptamer-QDs by one-pot could be applied for TomSys sensing based on GO.

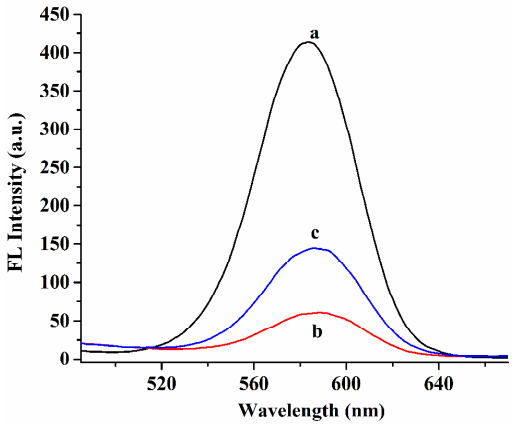

Figure 4. Fluorescence spectra of the biosensor with (a): aptamer-QDs Experimental conditions: aptamer-QDs, 26 nM; GO, 53.3 μg/mL; target TomSys, 1.17 μM.

3.5. Optimization of the reaction conditions

In order to achieve an optimal experimental result, the concentration of GO and different incubation time were optimized. As shown in Figure 5, as the concentration of GO increased from 0 to 93.4 μg/mL, the fluorescence intensity decreased gradually. The maximal quenching efficiencies were about 96% and the optimal concentration of GO was 53.3 μg/mL. Furthermore, different incubation time was investigated in this study (Figure S2), and the maximum change of fluorescence intensity (Δ F) was achieved at 15 min. Therefore, the most suitable incubation time of 15 min was selected.

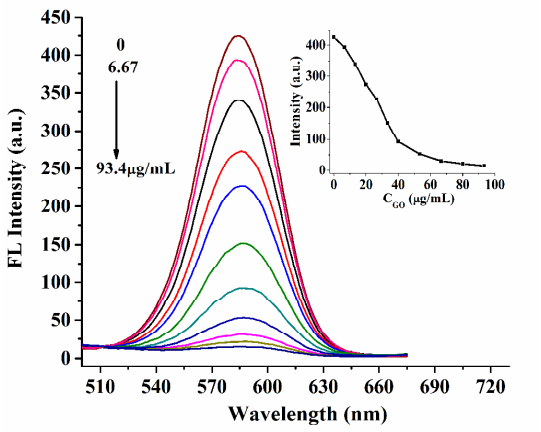

Figure 5. Fluorescence spectra of aptamer-QDs (26 nM) in the presence of different concentrations of GO. Inset: the curve of quenched fluorescence intensity of 586 nm aptamer-QDs upon different concentration of GO.

3.6. GO-based aptasensor for the detection of TomSys

Figure 6A showed the fluorescence signal with the different concentrations of TomSys under optimal experimental conditions. It could be seen that the fluorescence intensity increased gradually with the increasing concentration of TomSys. The plot of the ΔF value versus the concentration of the TomSys showed a good linear response in the range from 0.17 to 1.5 μM (Figure 6B), and the linear equation was y = 1.7490 + 69.3704x. The limit of detection (LOD) for TomSys was 0.1 μM based on the linear fitting and the noise level of 3 σ (where σ was the standard deviation of a blank solution, n = 9). The detection sensitivity of this method was significantly better than that of the previously reported aptasensor which constructed based on aptamer beacon [33].

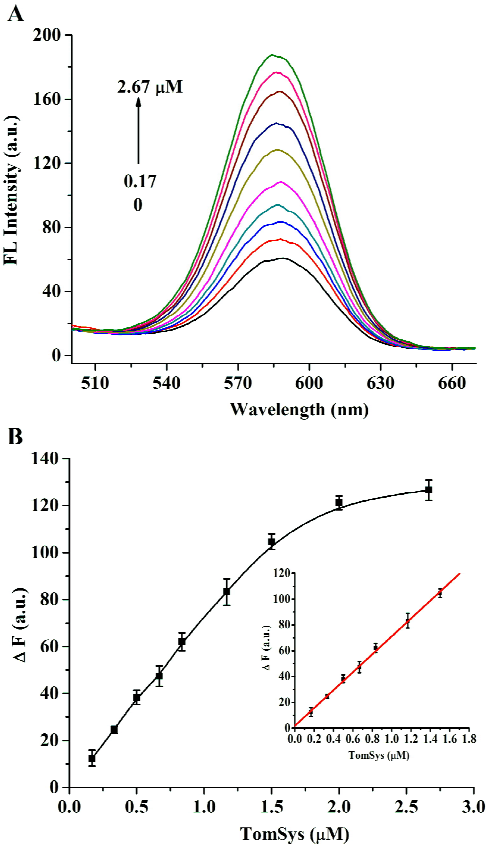

Figure 6. GO-based system for the detection of TomSys. (A) Changes in the fluorescence spectra upon different concentrations of the target TomSys: 0, 0.17, 0.33, 0.50, 0.67, 0.84, 1.17, 1.50, 2.00, and 2.67 μM (from the bottom to the top). (B) The linear curve of target TomSys detection. Inset: linear relationship between the ΔF (increased fluorescence intensity) and the concentrations of the target.

3.7. Selectivity analysis

According to the selectivity analysis procedure, the fluorescence responses of the sensor to BSA, Hb, and peptide were investigated. As shown in Figure 7, the nanosensor could effectively differentiate between TomSys and other targets. The fluorescence signal for the target TomSys was approximately three times higher than that of the BSA under the same concentration. This satisfactory selectivity was owing to the specificity between the aptamer and TomSys, and these results indicated the good selectivity of this platform.

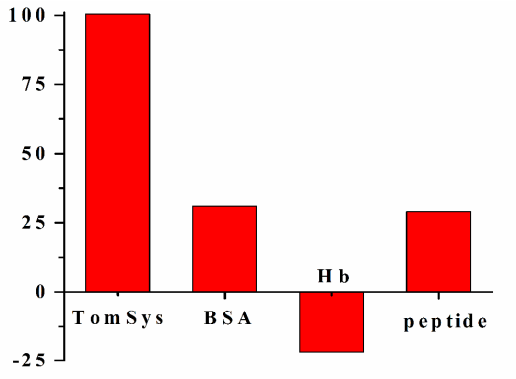

Figure 7. The change of fluorescence intensities of the sensing system toward different targets: TomSys, BSA, Hb, and peptide. The TomSys and other targets are all 1.17 μM.

3.8. Determination of target in plant tissue culture

To evaluate the application potential of the proposed biosensor in relatively complex biological matrix, a recovery test of TomSys in 10% plant tissue culture was performed. Different concentrations of TomSys were added into 10% plant tissue culture. The recovery percent of TomSys detected by the biosensor in plant tissue culture ranged from 92.1% to 118.0%, which was satisfactory for quantitative assays performed in biological samples (Table 1).

Table 1 Determination of target in 10% plant tissue culture with this biosensor

Sample number	Add (μM)	Found (μM)	Recovery %
1	0.233	0.275	118.0
2	0.500	0.506	101.2
3	0.835	0.769	92.1
4	1.167	1.123	96.2
5	1.333	1.426	107.0

4. Conclusions

In conclusion, a simple, specific and rapid fluorescent aptasensor based on GO is developed for the detection of TomSys. The aptamer-functionalized Zn^{2+} doped CdTe QDs have been synthesized through a facile one-pot hydrothermal route, which is simpler and cheaper than the conjugated DNA-QDs. The results indicate that aptamer-functionalized QDs can be successfully applied in a biosensor for the detection of TomSys, and the biosensing platform possesses satisfactory

sensitivity and selectivity. Therefore, the aptasensor of detecting TomSys based on GO hold the potential in chemical analysis and biological investigation of TomSys. In addition, it is anticipated that the aptamer-functionalized QDs may be applied to bind specifically to DNA, protein, and cell surface receptors based on aptamer recognition. In view of these advantages, the aptamer-functionalized QDs have great potential to paralleled analysis of multiple different targets.

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