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CdTe/CdSe quantum dot-based fluorescent aptasensor with hemin/G-quadruplex DNzyme for sensitive detection of lysozyme using rolling circle amplification and strand hybridization

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ABSTRACT

Lysozyme with a small monomeric globular enzymatic protein is part of the innate immune system, and its deficiency can cause the increased incidence of disease. Herein, we devise a new signal-enhanced fluorescence aptasensing platform for quantitative screening of lysozyme by coupling with rolling circle amplification (RCA) and strand hybridization reaction, accompanying the assembly of CdTe/CdSe quantum dots (QDs) and hemin/G-quadruplex DNzyme. Initially, target–triggered release of the primer was carried out from DNA duplex *via* the reaction of the aptamer with the analyte, and the released primer could be then utilized as the template to produce numerous repeated oligonucleotide sequences by the RCA reaction. Following that, the formed long-stranded DNA simultaneously hybridized with the CdTe/CdSe QD-labeled probe and hemin/G-quadruplex DNzyme strand in the system, thereby resulting in the quenching of QD fluorescent signal through the proximity hemin/G-quadruplex DNzyme on the basis of transferring photoexcited conduction band electrons of quantum dots to Fe(III)/Fe(II)-protoporphyrin IX (hemin) complex. Under optimal conditions, the fluorescent signal decreased with the increasing target lysozyme within the dynamic range from 5.0 to 500 nM with a detection limit (LOD) of 2.6 nM at the $3s_{blank}$ criterion. Intra-assay and interassay coefficients of variation (CVs) were below 8.5% and 11.5%, respectively. Finally, the system was applied to analyze spiked human serum samples, and the recoveries in all cases were 85–111.9%.

1. Introduction

Nucleic acid-based fluorescence quantitative analysis for microRNA (Hong et al., 2016; Liao et al., 2016; Chen et al., 2016, proteins/small molecules (Chen et al., 2016; Xue et al., 2012), and metal ions (Freage et al., 2014; Tan et al., 2016; He et al., 2014; Zhou et al., 2016) has emerged as a very useful technique in the past years, thus stimulating the fast development of the fluorescent aptasensors (Nakano et al., 2013; Ruckh et al., 2016; Liu et al., 2016; Kong et al., 2015) owing to their inherent advantages, *e.g.*, sensitivity, specificity, speed and economy. Despite some advances in this field, these approaches usually suffer from some unfavorable limitations, such as poor photostability and solubility in water, high cost, a complicated synthesis process and surface modification. In contrast, semiconductor nanocrystals or quantum dots (QDs) (Fernando et al., 2015; Zhou et al., 2015; Martín-Yerga et al., 2016; Shahmuradyan and Krull, 2016) are chosen as the valuable supplements relative to conventional fluorescent proteins (Duwé et al., 2015; Jablonski et al., 2013) and organic dyes (Venkateswararao et al., 2014), since they have the broad absorption spectra, size-tunable emission spectra, easy functionalization and strong photostability (Morales-Narváez et al., 2015; Pan et al., 2016; Samanta et al., 2012). Highly fluorescent quantum dots have shown promising applications in optical device (Bakulin et al., 2013), biological imaging (Allen et al., 2015), bioconjugate (Vinayaka et al., 2011), and optical biosensor (Huang et al., 2016). However, single-component quantum dots are easy to reunite because of surface defect. Core-shell quantum dot composites depending on selecting the core-shell materials can improve this phenomenon and endow some new properties.

Aqueous type-II core-shell CdTe/CdSe QDs have potential as important near-infrared fluorescent probes for drug screenings, biological imagings, and medical diagnostics due to low fluorescence background and high penetration of near infrared emission (Xia et al., 2008; Antanovich et al., 2015). Especially, the insoluble fluorescent probes are of the interests in the biological application because of low cost and low toxicity relative to the organic synthesis (Zhao et al., 2016). Moreover, the optical readout signals

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of the size-controlled quantum dots usually depend on the different photophysical mechanisms, such as fluorescence electron transfer quenching (Zhang et al., 2013), fluorescence resonance energy transfer (FRET) (Wu et al., 2015), and chemiluminescence resonance energy transfer (Lee et al., 2012). Freeman et al. (2011) reported a new detection system for small molecule and metal ion based on the self-assembly of hemin/G-quadruplex DNAzyme, aptamer and nucleic acid-modified quantum dots. Shahmuradyan and Krull devised intrinsically labeled fluorescent oligonucleotide probes on quantum dots for transduction of nucleic acid hybridization (Shahmuradyan et al., 2012). Sharon et al. utilized the interaction of CdSe/ZnS quantum dots with hemin/G-quadruplex for construction of luminescence aptasensor (Sharon et al., 2010). Further signal-amplified strategies have be developed coupling with quantum dots (Zeng et al., 2014; Zhao et al., 2015). Unfavorably, the detectable signals were always limited, especially using the single quantum dot. Rolling circle replication, a process of unidirectional nucleic acid replication, can rapidly synthesize multiple copies of circular molecules of DNA/RNA (Zhuang et al., 2014). In this case, a short DNA or RNA primer can form a long single-stranded DNA or RNA containing tens to hundreds of tandem repeats by using a circular DNA template and special polymerases (Liu et al., 2014). Compared with other signal-amplified strategies, rolling circle replication is capable of generating 10^{9-15}-fold signal amplification (Qian et al., 2003). To this end, our motivation in this work is to design a signal-amplified fluorescence detection platform based on the core-shell quantum dots and rolling circle amplification strategy.

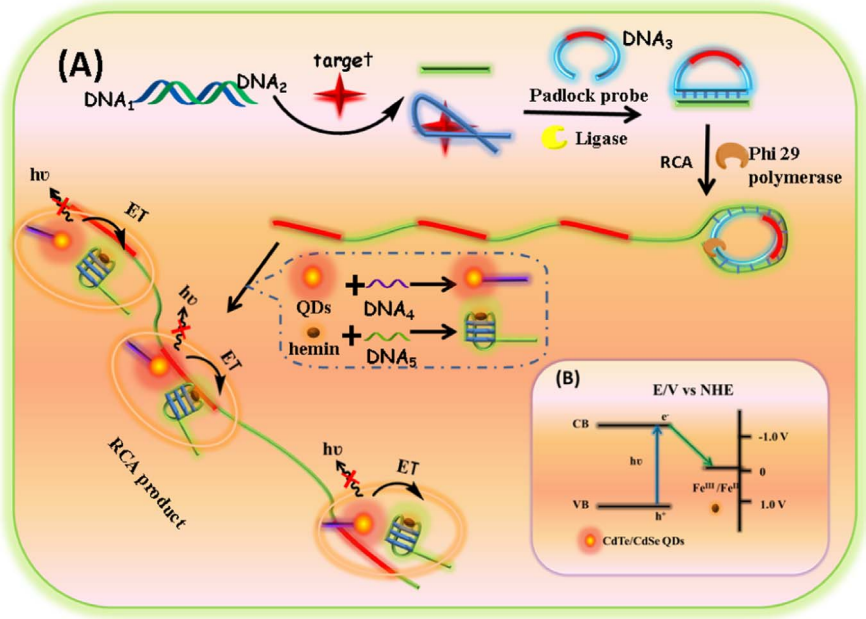
Lysozyme with a small monomeric globular enzymatic protein (glycoside hydrolases) is part of the innate immune system, and its deficiency can cause the increased incidence of disease. Elevated concentrations of lysozyme in serum/urine are related to meningitis, leukemia, and renal diseases (Huang et al., 2010). Herein, we report the proof-of-concept of feasible and *in-situ* amplified fluorescent aptasensor for the detection of lysozyme using CdTe/CdSe quantum dots and hemin/G-quadruplex DNzyme with rolling circle amplification (RCA)-based strand hybridization reaction (Scheme 1). Introduction of rolling circle replication is expected to enhance the conjugated amount of QD-based probes through the strand hybridization reaction, whilst hemin/G-quadruplex DNzyme is utilized to readily quench the fluorescent signal of quantum dots in the close proximity moieties. The released primer after the formation of the aptamer-target complex is used as the template for the RCA reaction with the help of ligase and polymerase. The quenching efficiency of free CdTe/CdSe quantum dots relies on the concentration of target lysozyme in the sample. During this process, the photoexcited conduction band electrons of quantum dots are transferred to the Fe(III)-protoporphyrin IX (hemin) complex (Shahmuradyan and Krull, 2016). Moreover, the lowest energy conduction band electrons of quantum dots are largely localized in the shell while the lowest energy valence band hole is localized in the core. This spatial distribution enables ultrafast electron transfer to the surface-adsorbed electron acceptors owing to the enhanced electron density on the shell, while simultaneously retarding the charge recombination process because the shell acts as a tunneling barrier for the core localized hole (Antanovich et al., 2015; Zhu et al., 2011; Chuang et al., 2010).

2. Experimental

2.1. Materials and reagents

Trisodium citrate dehydrate, 3-mercaptopropionic acid, Se powder, $Cd(NO_3)_2 \cdot 4H_2O$, Na_2TeO_3 and $NaBH_4$ were purchased from Sigma-Aldrich (St. Louis, MO 63103 USA). N-Hydroxysulfosuccinimide (NHS) and 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrochloride (EDC) were achieved from Alfa Aesar (Shanghai, China). Hemin and lysozyme were obtained from Sangon Biotechnol. Co., Ltd (Shanghai, China). The hemin stock solution (1.0 μM) was prepared in dimethyl sulfoxide (DMSO), and stored in darkness at 4 °C. T_4 DNA ligase, dNTP and Phi29 DNA polymerase were acquired from New England Biolab (Beijing, China). All the oligonucleotides were synthesized and purified by Sangon Biotechnol. Co., Ltd. (Shanghai, China). The sequences of the oligonucleotide used in this work are as follows:

DNA_1 (aptamer): 5′-ATCAGGGCTAAAGAGTGCAGAGTTACTTAG-3′.



Scheme 1. (A) Schematic illustration of CdTe/CdSe quantum dots (QDs)-based fluorescent aptasensing platform with hemin/G-quadruplex DNzyme for the ultrasensitive detection of lysozyme using rolling circle amplification (RCA) and strand hybridization reaction, and (B) the appropriate energy levels diagram of hemin/G-quadruplex DNzyme and CdTe/CdSe QDs associated with the electron transfer quenching mechanism.