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Cyclically amplified fluorescent detection of theophylline and thiamine pyrophosphate by coupling self-cleaving RNA ribozyme with endonuclease

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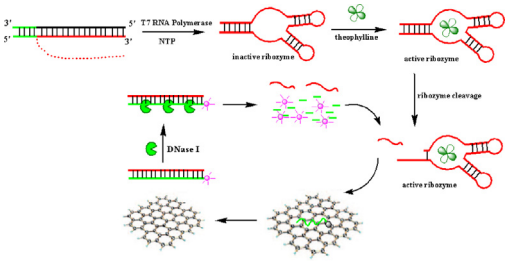
HIGHLIGHTS

- The RNA aptazymes were demonstrated as probe for the detection of theophylline and TPP.
- The higher fluorescence quenching efficiency of GO lead to the low background.
- The sensitivity was enhanced through the cyclic amplification induced by DNase I.

GRAPHICAL ABSTRACT

A structure-switching-based approach for the design of fluorescent biosensors from known RNA aptazymes were demonstrated for the detection of theophylline and TPP. Taking advantages of the ability of GO to protect ssDNA from nuclease cleavage and the cyclic amplification induced by DNase I, the assay was highly sensitive amplified.



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ABSTRACT

A structure-switching-based approach for the design of fluorescent biosensors from known RNA aptazymes were demonstrated for the detection of theophylline and thiamine pyrophosphate (TPP). Taking advantages of the ability of graphene oxide (GO) to protect ssDNA from nuclease cleavage and the cyclic amplification induced by deoxyribonuclease I (DNase I), the amplified assay showed high sensitivity. In the presence of target, the target-dependent hammerhead aptazyme cleaves off. The released Shine–Dalgarno (SD) sequence was introduced into the detection system, in which a FAM labeled probe ssDNA was noncovalently assembled on GO, and the fluorescence of the dye was completely quenched. In the presence of the released sequence, the binding between the dye-labeled DNA and the SD sequence alter the conformation of dye-labeled DNA, and disturb the interaction between the dye-labeled DNA and GO, liberating dye-labeled DNA from GO. The fluorescent intensity was increased, whereupon the DNase I can cleave the free DNA in the DNA/RNA complex, thereby liberating the fluorophore and ultimately releasing the SD RNA sequence. The released SD RNA sequence then binds another DNA probe, and the cycle starts anew, which leads to significant amplification of the fluorescent signal. The strategy showed good sensitivity and the dynamic ranges were of 0.1–10 μM and 0.5–100 μM for theophylline and TPP, respectively. The approach opens up a wide range of possibilities for sensing of other small molecules in biological entities.

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1. Introduction

Riboswitches are structured noncoding genetic elements existed widely in prokaryotic mRNAs that specifically bind small molecule metabolites and control gene expression [1,2]. Fast-cleaving variants of the hammerhead ribozyme (HHR) with stem I/stem II tertiary interactions have been used in eukaryotes for controlling gene expression. Artificial RNA-based switches have been generated by inserting aptamers into messenger RNA molecules, which then respond to the presence of the respective ligand with changes in gene expression [3,4]. They perform this function by changing their conformations upon binding to their specific ligands in the aptamer domain to promote or inhibit transcription termination, translation initiation, or splicing in the expression platform [5]. A self-cleaving HHR coupled with natural aptamer domains can create efficient ligand-dependent switches as a synthetic expression platform. A number of the artificial riboswitches act as genetic regulators in mammalian cell culture and animals as well as in bacteria [6–8]. For example, an aptamer specifically binding the theophylline was inserted into the mRNA of bacteria allowing eightfold inhibition of gene expression [9]. Win and Smolke developed a universal and extensible RNA-based platform that will provide a framework for the reliable design and construction of gene-regulatory systems, it can control the expression of specific target genes in response to various effector molecules [10].

These systems nicely demonstrate the potential of the artificial-riboswitch strategy as gene regulators in vivo. The analyte-dependent activity of aptazymes has enabled their use as biosensors for detection of various molecules [11]. Many artificial-riboswitch-based sensors have been developed [12–14]. Ogawa's group have reported some prokaryotic or eukaryotic riboswitch-based in vitro biosensors that were rationally designed using aptazymes, which are functional RNA and cleave themselves in response to their specific ligand molecules. The strategy used unique noncrosslinking gold nanoparticle aggregation or fluorescence resonance energy transfer (FRET) [15–17]. Savran et al. present the use of aptazymes as reagents for augmenting small ligand detection by quartz crystal microbalance (QCM) based on a mass-sensitive device [12]. Walter's group developed an assay for glucosamine 6-phosphate using a ligand-activated ribozyme with FRET and capillary electrophoresis-laser-induced fluorescence (CE-LIF) detection [18].

In these studies, various methods were used to detect the signal of ribozyme activity. However, in these assays, each aptamer binds to only a single target molecule and induced one cleavage. This 1:1 cleaving ratio limits signal enhancement and thus the sensitivity of the assay. To overcome this problem, the amplification techniques can be introduced. Amplification techniques have been developed for sensitive detection of DNA including polymerase chain reaction (PCR) [19], rolling circle amplification (RCA) [20], strand-displacement amplification (SDA) [21], hybridization chain reaction (HCR) [22]. In addition, the sensitive method for DNA detection based on endonuclease-induced amplification reaction was illustrated, which does not require a specific recognition site [23]. To the best of our knowledge, there are few reports on the combination of the signal amplification and target-dependent ribozyme for the detection of effector molecules.

To expand the exploration of artificial riboswitches in bioanalysis, we demonstrate a structure-switching-based approach for the design of fluorescent biosensors from known RNA aptazymes. We take advantage of the deoxyribonuclease I (DNase I) to create a signal-amplifying mechanism. DNase I is an endonuclease coded by the human gene DNASE1, that cleaves DNA preferentially at phosphodiester linkages adjacent to a pyrimidine nucleotide. It acts on single-stranded DNA, double-stranded DNA, DNA in DNA:RNA heteroduplexes, and chromatin. The use of theophylline is complicated by its interaction with various drugs, and that it has a narrow therapeutic index, so, as in the case with many other asthma drugs, its use must be monitored to avoid toxicity. Additonally, as most of these riboswitches have been demonstrated in bacteria with an aptamer for theophylline [24], theophylline was chosen as a model target. In the detection strategy, the DNA protection properties of graphene oxide (GO) nanomaterials and the amplification based on nuclease were involved.

2. Experimental

2.1. Apparatus

Fluorescence emission spectra were recorded on a F-4500 FL spectrophotometer (Tokyo, Japan) equipped with 1 cm quartz cells. The excitation was made at 480 nm with recording emission range of 500–600 nm. All excitation slits were set at 5 nm and emission slits were set at 10 nm. Polyacrylamide gel electrophoresis (PAGE) was performed in DYCZ-28C electrophoresis power supply equipped with WD-9413A gel documentation & analysis systems.

2.2. Chemicals

Double-strand DNA (dsDNA) templates designed according to the references [25,26] were synthesized using gene synthesis by Shanghai Generay Biotech Co. Ltd. FAM modified oligonucletides and T7 promoter used in the present study were purchased from Takara Biotechnology Co., Ltd (Dalian, China), and used without further purification. Their sequences were provided in Supplementary Table S1. Deoxyribonuclease I (DNase I), theophylline and thiamine pyrophosphate (TPP) were purchased from Sigma–Aldrich. Other chemicals employed were all of analytical grade and double distilled water was used throughout the experiments.

2.3. Preparation of graphene oxide

Graphene oxide was synthesized from the pre-oxidized graphite by a modified Hummers method [27]. Briefly, Graphite powder (2 g, 300 mesh) was added to a mixture of 12 mL concentrated H_2SO_4, 3.0 g $K_2S_2O_8$ and 3.0 g P_2O_5. The solution was stirred at 80 °C for 5 h. the pre-treated graphite was added to the concentrated H_2SO_4 (80 mL). Then $KMnO_4$ (10 g) was added gradually under stirring and the temperature of the mixture was kept to about 5 °C. Successively, the mixture was stirred at 35 °C for 4 h. Deionized was injected into the mixture followed by adding 30 mL 30% H_2O_2 drop by drop. The mixture was washed with 1:10 HCl aqueous solution and then DI water. Exfoliation was carried out by sonicating graphene oxide (2 mg mL^{-1}) dispersion in ice bath for 30 min. At last, the resulted sample was centrifuged at 12 k rpm for 10 min, and the upper solution was taken for future experiments. TEM image is shown in Fig. S1 in the supporting information.

2.4. RNA transcription and ribozyme cleavage

The RNA samples for the studies were generated by in vitro transcription using T7 RNA polymerase and synthetic DNA templates. The transcription reaction was conducted at 37 °C for 2 h in 150 μL of Transcription Buffer (40 mM Tris–HCl, pH 7.9, 6 mM $MgCl_2$, 10 mM DTT, 10 mM NaCl, 2 mM spermidine, 25 pmol dsDNA template, 2.5 mM each of GTP, CTP, UTP, ATP, 1.07 units μL^{-1} RiboLock Ribonuclease Inhibitor) and 1.33 units μL^{-1} T7 RNA polymerase (Fermentas). The reactions were stopped by addition of 1 volume of stop buffer (80% [v/v] formamide, 50 mM EDTA pH 8.0, 0.025% [w/v] bromphenolblue and 0.025% [w/v] xylene cyanole).

The resulting ribozyme was activated by heating with varying concentrations of theophylline to 72 °C for 2 min and then slowly

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