2010 International Conference on Nanotechnology and Biosensors
IPCBEE vol.2 (2011) © (2011) IACSIT Press, Singapore

Aptamer-based Biosensors through Target-induced Strand Release

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***Abstract*—Aiming to build simple and rapid fluorescent aptasensors with large signaling magnitude, we used a target-induced strand release strategy to transduce aptamer-target interactions into easily detectable signals. DNA aptasensors for IgE and vasopressin and RNA aptasensors for tobramycin and vasopressin were successfully developed. The binding of steroids with nuclease-resistant modified aptamers were also investigated.**

Keywords-aptasensors; target-induced strand release; nuclease-resistant modified aptamers

I. INTRODUCTION

Aptamers are short single-stranded oligonucleotides selected in vitro for specific, high-affinity binding to a broad range of molecular targets. Thanks to their unique characteristics and chemical structure, aptamers are promising candidates for use in biosensing and analytical applications[1,2]. There are several strategies to transduce aptamer-target interactions into easily detectable signals. Among them, target-induced strand release (also called structure-switching signaling) [3] is a desirable design strategy due to simple and rapid detection and large signaling magnitude. This strategy has been successfully used to develop fluorescent aptasensors for ATP[3,4], thrombin[3,4], cocaine[5,6], ochratoxin A[7], interferon-gamma[8], PDGF-BB[9] and theophylline[10]. These fluorescent aptasensor designs from target-induced strand release strategy are quickly being integrated into many signal-transduction techniques and have been proven to be powerful molecular tools. Here, we used this fluorescent sensor design strategy to develop DNA aptasensors for IgE and vasopressin, and RNA aptasensors for tobramycin and vasopressin. We also studied the binding of steroids with nuclease-resistant modified aptamers (phosphorothioate linkage, 2`-O-methyl RNA base, spiegelmer).

II. MATERIALS AND METHODS

A. Materials

Spiegelmers (L-enantiomeric oligonucleotides) were made and purified by ChemGenes (Wilmington, MA, USA). All other oligonucleotides were made and purified by Integrated DNA Technologies, Inc (Coralville, IA, USA). Human platelet-derived growth factor-BB (PDGF-BB), BSA, ATP, GTP, tobramycin, erythromycin A, deoxycorticosterone 21-glucoside and dehydroisoandrosterone 3-sulfate were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). L-vasopressin and D-vasopressin (mirror-image configuration of L-vasopressin, from D-amino acids) were custom-made by American Peptide Co., Inc (Sunnyvale, CA, USA). Immunoglobulin E (IgE) was from Athens Research & Technology (Athens, GA, USA). Cocaine and benzoyl ecgonine were obtained through the National Institute of Drug Abuse. Quant-iT^{TM} OliGreen ssDNA reagent was from Invitrogen (Eugene, OR, USA). Thiazole orange (TO) was obtained from Biochemika (distributed by Sigma-Aldrich Co.). The oligonucleotides used, IgE aptamer-F: F-ATC TCA CGT TTA TCC GTC CCT CCT AGT GGC GTG A; IgE-Q: GTG GAC GTG AGA T-Q; PDGF-BB aptamer-F: F-ATC ACG GCA CAG GCT ACG GCA CGT AGA GCA TCA CCA TGA TCC TGT GC; PDGF-BB-Q: CCT GTG CCG AGA T-Q; Vasopressin DNA aptamer-F: F-TTA TCA CGT GCA TGA TAG ACG GCG AAG CCG TCG AGT TGC TGT GTG CCG ATG CAC GT; Vasopressin DNA-Q: ATG CAC GTG ATA A-Q; Tobramycin aptamer-F (all RNA bases): F-CCA UCG GGU AGA CUU GGU UUA GGU AAU GAG UCU ACC C; Tobramycin-Q(all DNA bases): AGT CTA CCC GAT GG-Q; Vasopressin RNA aptamer-F (all RNA bases): GGG GUA GGG CUU GGA UGG G(dT-F)A GUA CAC (PEG18) GUG UGC GUG GU; Vasopressin RNA-Q (all DNA bases): Q-CCA TCC AAG C; Vasopressin RNA aptamer (all RNA bases): GGG GUA GGG CUU GGA UGG GUA GUA CAC (PEG18) GUG UGC GUG GU; ATP DNA aptamer: ACC TGG GGG AGT ATT GCG GAG GAA GGT; Cocaine aptamer: GGG AGA CAA GGA TAA ATC CTT CAA TGA AGT GGG TCG ACA. dT-F: fluoresceinated dT base; PEG18: hexaethylene glycol spacer; F: fluorescein; Q: dabcyl.

B. Measurements

All measurements were performed on a Perkin-Elmer Victor II microplate reader (Shelton, CT, USA) in the specific selection buffers. A mixture of aptamer strand (fluorescein labeled) and quencher strand (dabcyl labeled) was incubated for 5 min at room temperature or 37 ºC (only for vasopressin RNA aptamer), then a series of standard dilutions of analytes were added to the mixture. Measurements were performed after 30 min. For the interaction of TO, OliGreen dyes with aptamers (without labeling), dyes were mixed with aptamers and incubated for 30 min, then analytes were added for 30 min to displace dyes. For IgE and PDGF-BB, the binding buffer of 10mM PBS, pH 7.4, 140mM NaCl, 2.7 mM KCl, 1mM $MgCl_2$ was used.

For vasopressin and tobramycin, the binding buffer of 20mM Tris-HCl, pH 7.4, 140mM NaCl, 5mM KCl, 1mM $MgCl_2$, 1 mM $CaCl_2$ was used. For ATP, the binding buffer of 20mM Tris-HCl, pH 7.6, 300mM NaCl, 5mM $MgCl_2$ was used. For cocaine and steroids, the binding buffer of 20mM Tris-HCl, pH7.4, 140mM NaCl, 5mM KCl, 2 mM $MgCl_2$ was used.

III. Results And Discussion

A. Aptasensors based on target-induced strand release

In a typical target-induced strand release strategy, aptamer specific to the target serves as the molecular recognition element, and is labeled with a fluorophore dye. The competing short oligonucleotide is complementary to the fluorophore labeled side of the aptamer, and is labeled with a quencher. The pre-formed duplex between a fluorophore-labeled aptamer and a quencher-labeled short oligonucleotide brings the dye close to the quencher so that there is low fluorescence. However, in the presence of target, the quencher-labeled strand is released from aptamer by target binding, which is accompanied by an increase of the fluorescent signal by dequenching of the fluorophore [3] (Fig. 1a). The molecular beacon (a hairpin oligonucleotide with a fluorophore attached on one end and a quencher attached on the other end) was also used as the competing oligonucleotide to develop the signal-off detections [4,11].

We first tested the reported DNA aptamer for IgE [12] in the buffer in which selection was performed. The efficient release of the quencher strand by IgE protein in low nM range is consistent with the strong interaction between IgE and the aptamer. We also demonstrated that the aptamer preserves it`s strong selectivity for IgE over BSA (Fig. 1b). Sung et al [9] used quantum dot as fluorescent donor to detect PDGF-BB[13]. We used a simpler organic dye to label PDGF-BB aptamer, the results also show that the aptamer preserves its strong sensitivity and selectivity (Fig. 1c). For vasopressin peptide (D-form)[14], the results show that vasopressin can compete efficiently with the quencher strand to release it from its DNA aptamer (Fig. 1d).

There is only one report describing structure-switching signaling strategy for RNA aptamers [10]. We tested one RNA aptamer for tobramycin [15] using this signaling strategy, and the results show that the aptamer has good response with tobramycin, and no response with erythromycin A (Fig. 1e). Vasopressin RNA aptamer [16] is selected against D-form vasopressin at 37 ºC, and we tested its binding with D- and L-form vasopressins at 37 ºC. Only the D-form has good response (Fig. 1f). We used aptamer-based thiazole orange (TO) displacement assay[17,18] to further investigate the binding of vasopressin and its RNA aptamer. This assay does not need any labeling. The aptamer binds to TO dye and results in a fluorescent complex. The presence of analyte in solution results in the displacement of dye from the complex and a reduction in fluorescence (Fig. 2a). Two forms of vasopressin were tested (Fig. 2b), and the responses in different temperatures were also compared (Fig. 2c). Another dye, OliGreen[19], was also tested for the ATP DNA aptamer[20] and cocaine aptamer [21] (Fig. 2d).

To get a successful target-induced strand displacement design, several important steps have to be followed. The aptamer needs to be labeled with a fluorophore at a site that will not kill the binding. The competing quencher strand should be long enough to form a relatively stable duplex with the aptamer so that the fluorescence is low in the absence of the target. However, the duplex should not be too stable to allow the release of the quencher strand by target binding[3]. Also, the domain of the aptamer for quencher strand binding should be in the vicinity of the binding pocket so that the binding-induced conformation change could release the quencher strand. For each aptamer, several fluorophore strands and quencher strands were tested to optimize the design.

B. The post-SELEX modifications of aptamers for steroids

Wild-type RNA and DNA aptamers are susceptible to degradation by nucleases. This will limit their applications for real samples, such as patient blood. Resistance to nuclease degradation can be greatly increased by the incorporation of phosphorothioate linkages, 2`-O-methyl nucleotides, and spiegelmers. We used the target-induced strand release design to study the steroid binding of the asymmetric three-way junction aptamers[22] (originally selected for cocaine[21]) by incorporating these modifications. The results show that these modified aptamers still preserve their binding capacities with steroids (Fig. 3).

Acknowledgment

The author is grateful to Dr. Milan N. Stojanovic and Dr. Donald W. Landry for support of this work. This work was supported by the US National Science Foundation (NSF) to M.N.S. and Office of Naval Research (ONR) to D.W.L. and M.N.S.

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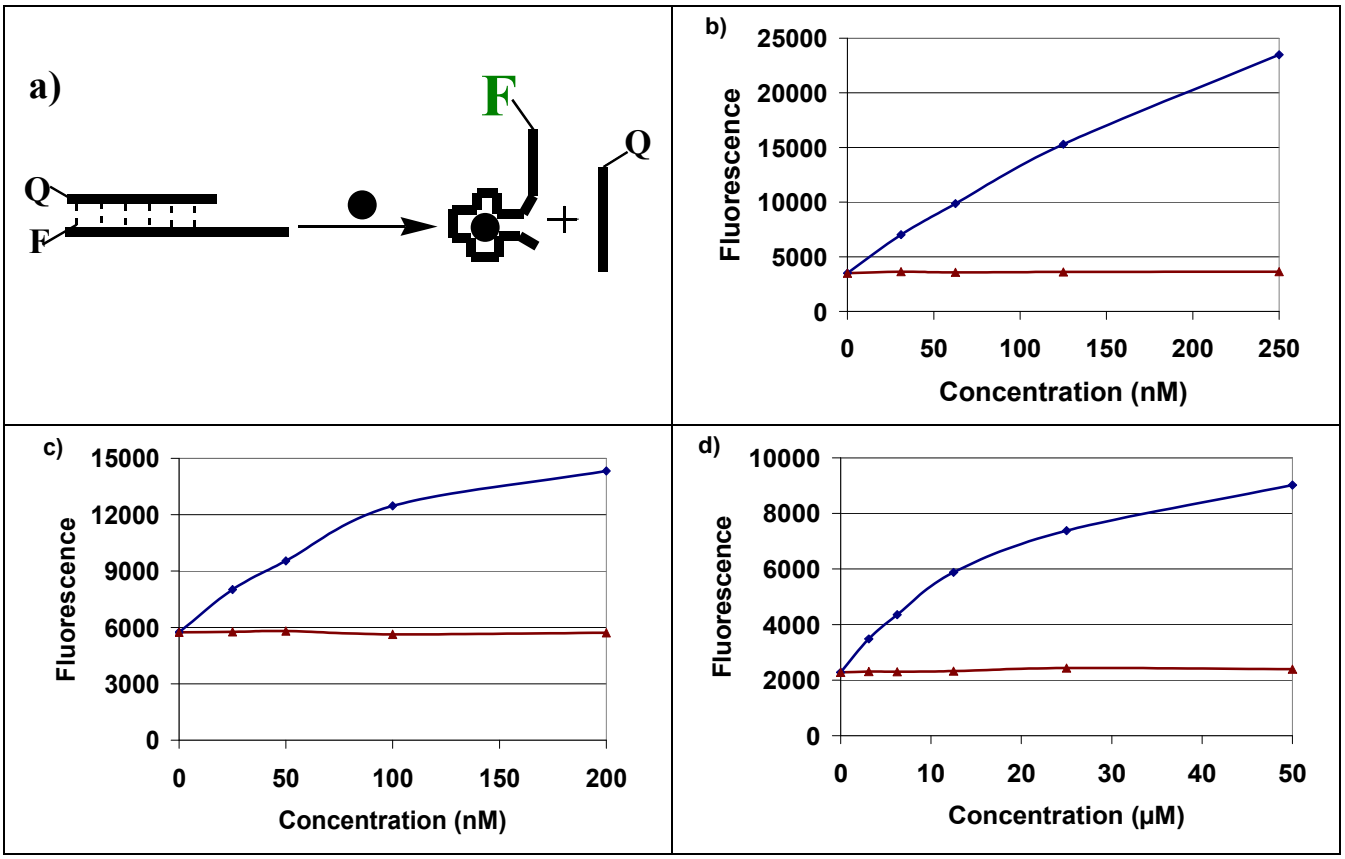

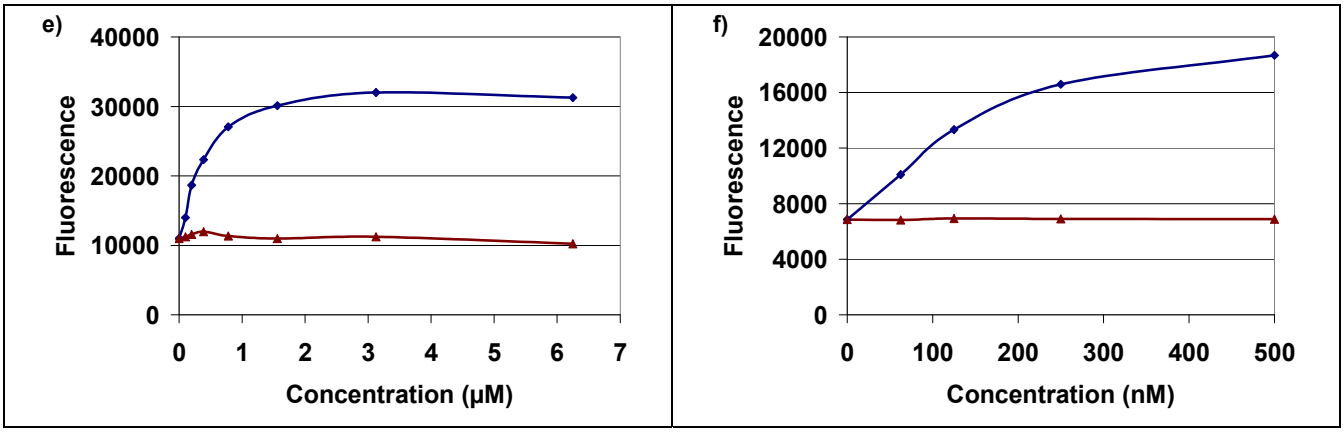

Figure 1. Aptasensors based on target-induced strand release. (a) Principle for converting aptamers into fluorescent sensors. A fluorophore labeled aptamer is complexed with a short complementary quencher labeled strand with a low fluorescence. In the presence of the target (black circle), the binding of target with aptamer induces the conformational change of aptamer to release the quencher strand, leading to a high fluorescence. (b) Fluorescence response of the mixture of IgE aptamer-F (50nM) and IgE-Q (150nM) in the presence of various concentrations of IgE (blue diamond) and BSA (red triangle). (c) Fluorescence response of the mixture of PDGF-BB aptamer-F (50nM) and PDGF-BB-Q (150nM) in the presence of PDGF-BB (blue diamond) and BSA (red triangle). (d) Fluorescence response of the mixture of vasopressin DNA aptamer-F (50nM) and vasopressin DNA-Q (150nM) in the presence of D-vasopressin (blue diamond) and L-vasopressin (red triangle). (e) Fluorescence response of the mixture of tobramycin aptamer-F (50nM) and tobramycin-Q (150nM) in the presence of tobramycin (blue diamond) and erythromycin A (red triangle). (f) Fluorescence response of the mixture of vasopressin RNA aptamer-F (100nM) and vasopressin RNA-Q (300nM) in the presence of D-vasopressin (blue diamond) and L-vasopressin (red triangle) at 37 ºC.

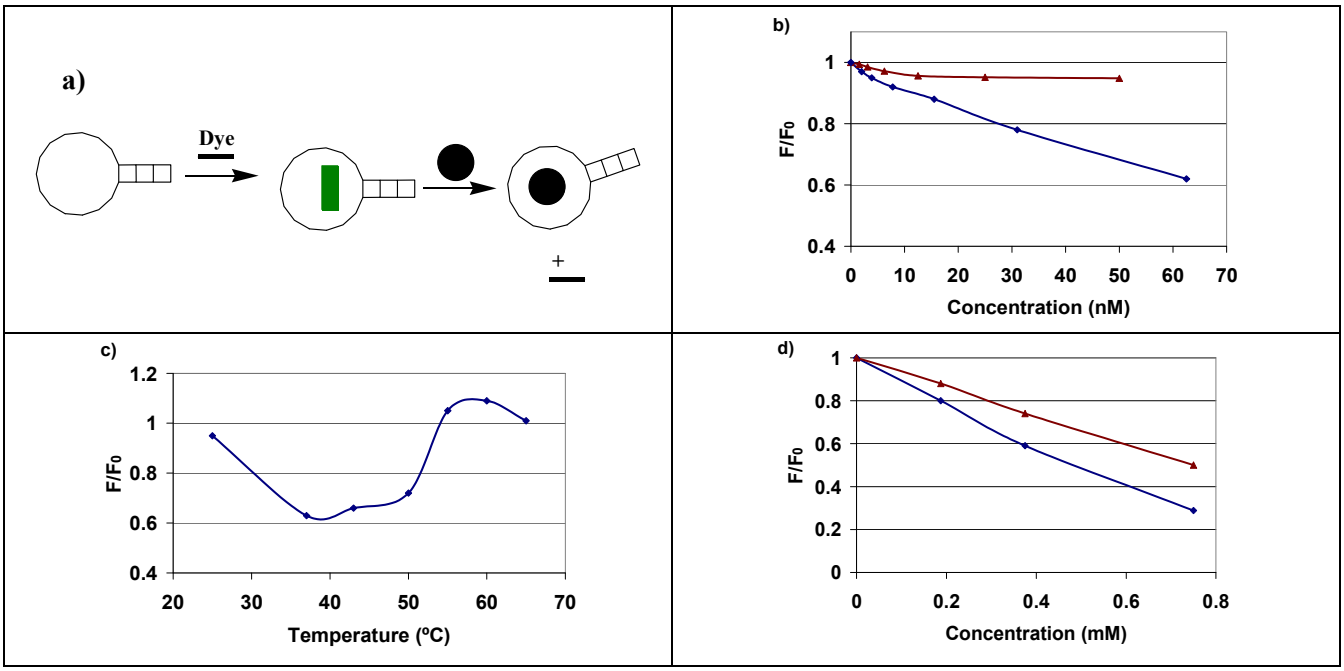

Figure 2. Aptamer-based dye-displacement assays. (a) Principle behind aptamer-based dye-displacement assays. A non-labeled aptamer binds to dye (TO or OliGreen) resulting in a fluorescent complex. The presence of target molecules (black circle) results in the displacement of dye from the complex and a reduction in fluorescence. (b) Fluorescence response of the mixture of TO (50nM) and vasopressin RNA aptamer (50nM) in the presence of D-vasopressin (blue diamond) and L-vasopressin (red triangle) at 37 ºC. (c) Temperature dependence of binding of D-vasopressin (63nM) with vasopressin RNA aptamer (50nM) to release TO (50nM) from the complex. (d) Fluorescence response of the mixture of OliGreen (1:3200 dilution) and ATP DNA aptamer (50nM) (blue diamond) or cocaine aptamer (50nM) (red triangle). The background fluorescence signal F0 was measured for the complex of aptamer and dye. F was measured in the presence of various concentrations of target molecules.

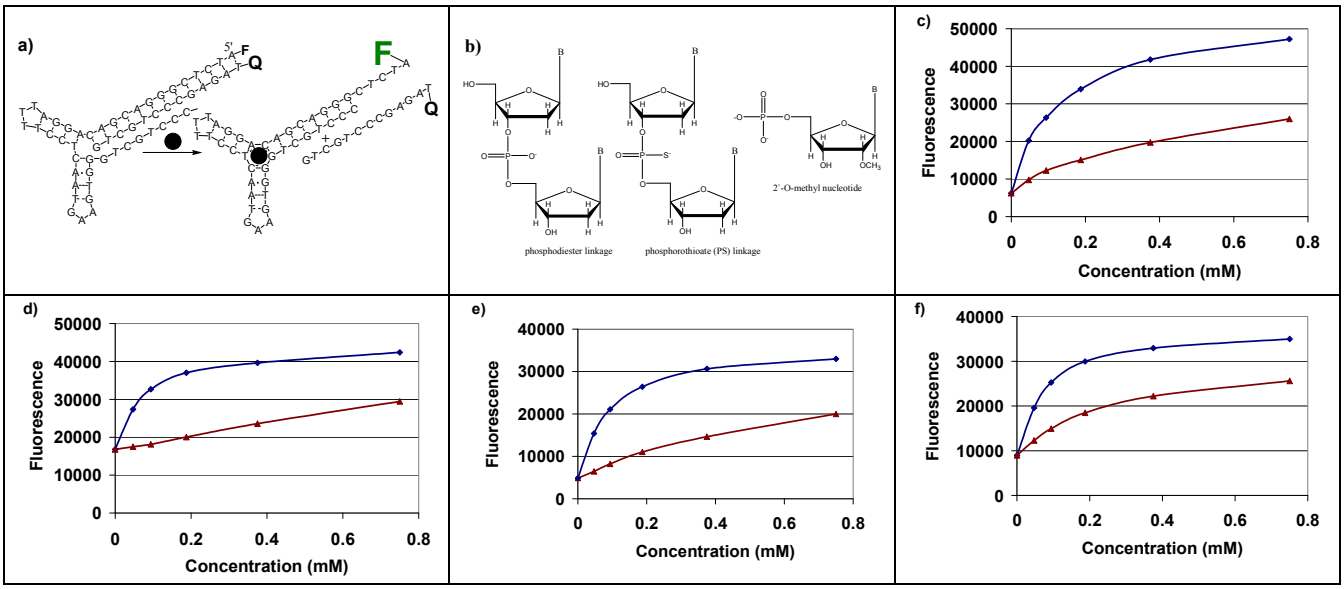

Figure 3. Steroid binding of the modified asymmetric three-way junction aptamers. (a) Structure of asymmetric three-way junction for target-induced strand release. (b) Structures of phosphodiester linkage (native), phosphorothioate (PS) linkage and 2`-O-methyl nucleotide. (c) Fluorescence response of the mixture of fluorescein labeled asymmetric three-way junction (all native phosphodiester linkages) (50nM) and its quencher strand (all native phosphodiester linkages) (150nM) in the presence of deoxycorticosterone 21-glucoside (blue diamond) and dehydroisoandrosterone 3-sulfate (red triangle). (d) 50nM phosphorothioate linkage junction and its phosphorothioate linkage quencher strand (500nM). (e) 50nM 2`-O-methyl nucleotide junction and its 2`-O-methyl nucleotide quencher strand (150nM). (f) 50nM spiegelmer junction and its spiegelmer quencher strand (150nM).