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Enzymatic cleavage and mass amplification strategy for small molecule detection using aptamer-based fluorescence polarization biosensor

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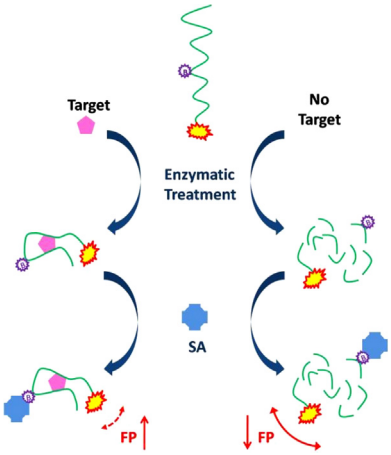
HIGHLIGHTS

- A novel mass amplification strategy was developed.
- This strategy can be used for sensitive detection of small molecule in complex biological samples.
- Excellent performances in adenosine measurement with aptamer-based FP probe.

GRAPHICAL ABSTRACT



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ABSTRACT

Fluorescence polarization (FP) assays incorporated with fluorophore-labeled aptamers have attracted great interest in recent years. However, detecting small molecules through the use of FP assays still remains a challenge because small-molecule binding only results in negligible changes in the molecular weight of the fluorophore-labeled aptamer. To address this issue, we herein report a fluorescence polarization (FP) aptamer assay that incorporates a novel signal amplification strategy for highly sensitive detection of small molecules. In the absence of adenosine, our model target, free FAM-labeled aptamer can be digested by nuclease, resulting in the release of FAM-labeled nucleotide segments from the ***dT***-biotin/streptavidin complex with weak background signal. However, in the presence of target, the FAM-labeled aptamer–target complex protects the FAM-labeled aptamer from nuclease cleavage, allowing streptavidin to act as a molar mass amplifier. The resulting increase in molecular mass and FP intensity of the aptamer–target complex provides improved sensitivity for concentration measurement. The probe could detect adenosine from 0.5 μM to 1000 μM, with a detection limit of 500 nM, showing that the sensitivity of the probe is superior to aptamer-based FP approaches previously reported for adenosine. Importantly, FP could resist environmental interferences, making it useful for complex

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biological samples without any tedious sample pretreatments. Our results demonstrate that this dual-amplified, aptamer-based strategy can be used to design fluorescence polarization probes for rapid, sensitive, and selective measurement of small molecules in complicated biological environment.

1. Introduction

Fluorescence polarization (FP) is a promising choice for fluorescence sensing based on fast, accurate and sensitive signal reporting [1–3]. Moreover, as a ratiometric approach, FP is closely related to its rotational relaxation time, which, in turn, depends on its molecular volume (molecular weight) under constant temperature and solution viscosity [4,5]. Furthermore, FP is insensitive to fluorescence fluctuation and photobleaching, and it can be directly used in complex biological environment. During the past few decades, FP assays have been successfully employed in many research areas, such as protein–protein interaction, protein–DNA interaction and immunoassays for drug discovery, diagnosis, food analysis, and environmental monitoring [6–8].

The emergence of aptamer probes has broadened the use of FP assays in many research areas. Typically, the binding of dye-labeled aptamer to ligand proteins, or any other macromolecules, results in large mass change from free aptamer to aptamer–target complex, allowing FP probes to successfully sense macromolecule dynamics, including, for example, the real-time monitoring of oncogenic protein platelet-derived growth factor [9]. Furthermore, aptamer-based FP has also been widely used for detecting various targets, such as proteins and cancer cells [10–12]. To construct an effective FP sensor, the target molecule should bring change of molecular mass from free probe to target probe. In this sense, binding with a small target would only generate negligible change in molecular weight and global rotation between free fluorophore-labeled probe and fluorophore-labeled probe–target complex, making FP unsuitable for the detection of small molecules.

However, with elegant design based on the unique conformational flexibility of aptamers, some aptamer-based FP probes for small-molecule detection have recently been proposed using competitive displacement, induced-fit binding, and mass amplification strategies [13–16]. Among them, mass amplification using proteins or nanomaterials as amplification moieties has been widely developed based on simple design and effective signal output. For example, Zhu et al. [17] and Cui et al. [18] presented some simple methods to transduce aptamer/small-molecule binding into a detectable signal based on fluorescence anisotropy, as well as allosteric probes, using thrombin or single-stranded DNA binding (SSB) protein as the mass amplifier, respectively. Nonetheless, by their facile deactivation, most proteins are hard to detect and thus unsuitable for practical applications. To address this problem, our group reported a fluorescence anisotropy (FA) signal amplification strategy to sensitively detect small molecules in real time by employing graphene oxide as an amplifier [19]. In addition to mass amplification strategies, the unique induced-fit mechanism of aptamers has also been proposed as an alternative scheme to develop FP biosensors for small-molecule detection [20]. For example, Kidd et al. introduced a method of target-triggered enzymatic cleavage protection in which the absence of target results in the digestion of the aptamer by nuclease, while the presence of target protects the aptamer from digestion by nuclease. Such intact aptamer then allows for relatively higher FP intensity compared to that of cleaved aptamer [21]. All of these methods combine aptamer/target binding with an amplification moiety, making small-molecule detection achievable with FA assays.

Phosphodiesterase I (PDE I) is an exonuclease that successively hydrolyzes 5′-nucleotides from the 3′-hydroxy-termini of both single-stranded and double-stranded DNAs. In our design, PDE I enzymatically cleaved the DNA aptamer substrate in the absence of target molecule, and then released mononucleotides/short single-stranded DNA fragments. The enzymatic cleavage was predicted to be impeded by binding of specific analyte to the functional DNA, resulting in the protection of the aptamer structure. Therefore, target-triggered enzymatic cleavage protection has been combined with streptavidin, as a molar mass amplifier, to produce a dual-amplification strategy that sensitively detects small molecules in homogeneous solution using the FP assay. In the absence of target, nuclease freely and efficiently digests the unbound aptamer into small DNA segments, including dye-labeled DNA segments and biotin-labeled DNA segments, resulting low FP signal of the dye-labeled small DNA segments. However, in the presence of target, the aptamer/target binding complex prevents aptamer cleavage, by conformational or structural changes or by steric hindrance [22,23]. Next, after binding of biotin to the mass amplifier, streptavidin, FP intensity of the aptamer–target complex significantly increases, and dual-amplified detection of small molecules is achieved. As a proof-of-concept, we chose adenosine as the model molecule to demonstrate the feasibility of target-triggered enzymatic cleavage protection and DNA–protein mass amplification, and a detection limit (LOD) of 500 nM was achieved. More importantly, this FP assay was applied to directly detect 3 μM of adenosine in complex biological samples based on its ability to resist environmental interferences. Therefore, this work demonstrates a simple and sensitive aptamer-based FP assay for small-molecule detection, which can be achieved by combining aptamer enzymatic cleavage protection with mass amplification.

2. Materials and methods

2.1. Materials and instrumentation

Streptavidin, adenosine, cytidine, uridine, guanosine and PDE I type IV from *Crotalus atrox* (PDE I) were purchased from Sigma–Aldrich Company (Shanghai, China). RPMI 1640 medium (GIBCO) supplemented with 10% fetal bovine serum (FBS) (heat inactivated, GIBCO) was used as the cell medium. All solutions were prepared in Milli-Q water (resistance >18 MΩ cm) from a Millipore system. Fluorescence measurements were performed on a FluoroMax-4 spectrofluorometer (Horiba Jobin Yvon, France) with L-format configuration at room temperature. Excitation and emission monochromators were set at 488 nm and 520 nm with 5 nm bandwidth, respectively. All the DNA oligonucleotides were synthesized and purified by Sangon Biotechnology Co., Ltd. (Shanghai, China). The sequence of aptamer-adenosine (Apt-A) was 5′-AG***dT*** GAA CCT GGG GGA GTA TTG CGG AGG AAG GT-3′ with fluorescein dye at the 5′-end and ***dT*** labeled by biotin. The sequence of aptamer-adenosine-1 (Apt-A-1) was 5′-AGT GAA CCT GGG GGA GTA TTG CGG AGG AAG GT-3′ with fluorescein dye at the 5′-end. Tris-buffer solution (20 mM Tris–HCl, pH 7.5, 20 mM $MgCl_2$, 10 mM NaCl) was used throughout the experiments.

2.2. Fluorescence measurements

Various PDE I solutions ranging from C_0/500 to C_0/1500 were prepared by dilution of the C_0 solution (2 units mL^{-1}) in Milli-Q