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Research Paper

Aptamer adaptive binding assessed by stilbene photoisomerization towards regenerating aptasensors

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ABSTRACT

Fluorescent aptasensors are reliant on static fluorescence intensity measurements, which suffer drawbacks such as background interference and laborious separation procedures. A unique aptasensor based on the photochrome aptamer switch assay (PHASA) has been developed that is independent of background fluorescence, requires no analyte separation, and allows rapid quantification within seconds. Malachite green aptamer (MGA) conjugated with a water-soluble stilbene on the MGA 3′ C38 terminus was chosen for building the proof-of-concept aptasensor. In the presence of malachite green and tetramethylrosamine ligands, the rate of the stilbene fluorescence decay was found to be linearly dependent on the ligand concentration. Molecular dynamic simulation suggests hydrogen bonding between stilbene sulfonates and neighboring nucleotides is the primary mechanism responsible for rate changes in stilbene photoisomerization. Analysis of the apparent fluorescence decay rate (k_{app}) versus analyte concentration gives a limit of detection (LOD) of 2 μM for MG and 0.6 μM for TMR. This aptasensor design opens up a new sensing mode, which is promising for rapid development of SELEX generated molecular recognition elements.

1. Introduction

Aptamers are synthetic single-stranded oligonucleotides obtained by systematic evolution of exponential enrichment (SELEX) technology in vitro, which can bind to various targets with extremely high specificity, for instance, drugs, growth factors, peptides, enzymes, spores, toxins, proteins, whole cells and ions [1–6]. As candidates for replacing antibodies, aptamers possess advantages of relatively simple synthetic preparation, low batch-to-batch variability, facile modification, low immunogenicity, and structural robustness [7–10]. In addition, it has been widely reported that aptamers have unique properties of conformational change/adaptive binding, making them more versatile as molecular recognition elements [11–15]. Thus, they have been applied in diagnosis, biosensing, bio-imaging, drug delivery, and drug discovery [16–18]. Electrochemical, fluorescent, chemiluminescent, and colorimetric sensors have employed aptamers for their molecular recognition properties. [16,19,20]. Among these sensors, fluorescent aptasensors are under rapid development, but largely rely upon static fluorescence intensity and fluorescence quantum yield measurements. [21]. While simple and straightforward, static fluorescence measurements are susceptible to fluorescence background interference and may require additional washing protocols to increase sensitivity.

Stilbene (1,2-Diphenylethene) fluorophores have been employed as fluorescence probes in our previous work but never in aptasensors [22–28]. This is mainly because of their strong tendency to lose fluorescence rapidly upon excitation via the non-radiative deactivation process connected to the twisted transition in the excited state [25,29]. The latter is responsible for the *trans-cis* photoisomerization of the molecule and acts as a quenching funnel on fluorescence emission, thereby depriving the

Abbreviations: MG, malachite green; MGA, malachite green aptamer; ITC-Stilbene, 4-Acetamido-4'- isothiocyanato-2,2'-stilbenedisulfonic acid disodium salt; RhB, rhodamine B; TMR, tetramethylrosamine; PHASA, photochrome aptamer switch assay.

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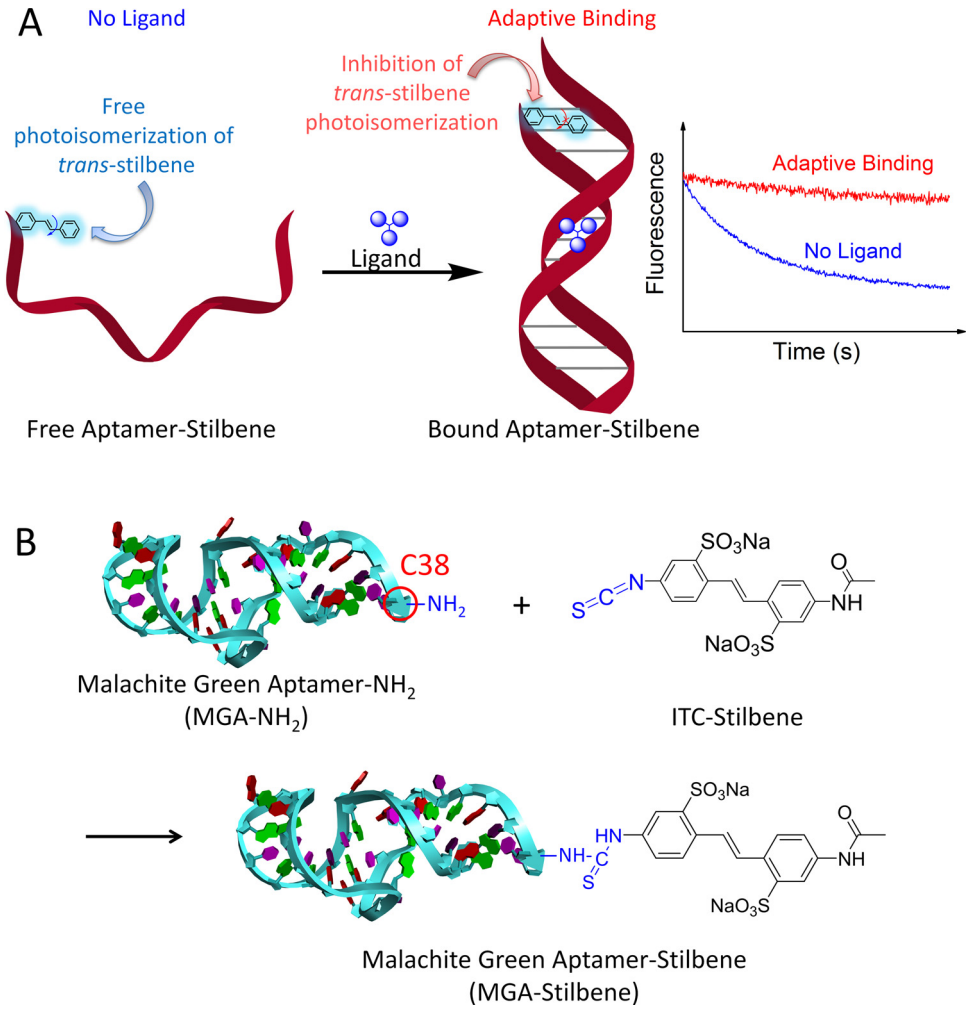

Fig. 1. (A) Schematic representation of the PHASA and related sensing application based on inhibition of the stilbene fluorescence decay due to aptameric conformational change. In the absence of ligand (analyte), *trans*-stilbene grafted on aptamer undergoes free photoisomerisation (fluorescent *trans*-isomer is converted to non-fluorescent *cis*-isomer under irradiation with excitation light), resulting in fast fluorescence decay. Upon binding to ligand (analyte), conformational change of aptamer induces the inhibition of *trans*-stilbene photoisomerisation, resulting in slowing of the fluorescence decay. (B) Synthesis of malachite green aptamer-stilbene (MGA-Stilbene) conjugate (see Fig. S1 for details of C38-C6-NH_2 modification). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

fluorescence probe of its photochemical stability. Under constant illumination at excitation maximum, the sterically unhindered *trans*-stilbene molecules in solution rapidly change their molecular configuration with observed fluorescence emission decay. The fluorescence decay kinetics is strongly dependent upon local steric and electronic environments. [23,30–32]. Such photochemical instability is detrimental if the stilbene compound is considered a classical fluorescence probe, and this is probably the reason why stilbene compounds have never been effectively used as fluorescent reporters (labels or probes) in biosensors. However, the actual reporting power of the stilbenes is not in their fluorescence, but in a rapid loss of their fluorescence via an instant conformational change upon excitation. This makes the stilbene switches unique in the sense that most fluorescent reporters either do not possess this intramolecular switchable nature or require the separation of adjacent fluorophores.One of the most striking features of stilbene photochemistry is its essentially strong dependence on local environments, which can effectively alter or even hinder the photoisomerization of the molecule in the excited state. [27,33,34]. This includes grafting of stilbene compounds on oligonucleotides [35].

In this proof-of-concept work, we report a new molecular sensor based on stilbene fluorophores conjugated to an RNA aptamer molecular recognition element. Photochrome aptamer switch assay (PHASA) concept, design and preparation of the elements, and the long-term stability of the RNA aptamer have previously been reported [26,36,37]. Our previous investigation focused on the synthesis of stilbene maleimide derivatives and prepared an aptamer-stilbene based on maleimide-thiol conjugation. However, the poor synthetic yields and low emission intensities of the synthesized conjugates prevented a quantitative ligand binding analysis. In our current study, we successfully constructed MGA-Stilbene conjugate based on amine-isothiocyanate grafting that attained an 80% synthetic yield with adequate S/N emission intensities. For the first time, the PHASA concept is tested and successfully demonstrated, which presents a novel fluorescence decay-based sensing mode. The PHASA-based 'aptasensor' detects ligand induced adaptive binding based on changes in the apparent rate of the stilbene fluorescence decay under constant-illumination conditions, which forms our central hypothesis. This proof-of-concept work (Fig. 1A and B) incorporates four unique features towards the synthesis and exposition of the PHASA elements: 1) MGA's (malachite green aptamer) ability to undergo adaptive binding (structural folding around ligand), 2) chemical synthesis of MGA that allows incorporation of reactive functional groups, 3) sensitivity of stilbene's photoisomerization kinetics to sterical