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Facile conversion of ATP-binding RNA aptamer to quencher-free molecular aptamer beacon

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ABSTRACT

We have developed RNA-based quencher-free molecular aptamer beacons (RNA-based QF-MABs) for the detection of ATP, taking advantage of the conformational changes associated with ATP binding to the ATP-binding RNA aptamer. The RNA aptamer, with its well-defined structure, was readily converted to the fluorescence sensors by incorporating a fluorophore into the loop region of the hairpin structure. These RNA-based QF-MABs exhibited fluorescence signals in the presence of ATP relative to their low background signals in the absence of ATP. The fluorescence emission intensity increased upon formation of a RNA-based QF-MAB·ATP complex.

Adenosine-5'-triphosphate (ATP) is an energy storage molecule that regulates several important biological processes in living cells, including muscle transportation, and biomolecule synthesis and degradation.[1] The detection of ATP is fundamental aspect of many clinical diagnoses and biochemical studies. Several strategies for ATP detection have been developed using DNA-based ATP aptamers with fluorescence methods.[2]

Molecular beacons (MBs) have been the most common oligonucleotide probes; they are typically designed to feature a hairpin structure, with the stem part labeled with a fluorophore on one end and a quencher on the other.[3] The change in fluorescence of an MB results from the conformational change in the loop region induced through hybridization with the target. Recently, the signal transduction of MBs has been combined with the binding affinity of aptamers to develop novel oligonucleotide probes—so-called molecular aptamer beacons (MABs)—that have expanded the range of utility of classical MBs to the detection of non-nucleic acid targets.[4] Although MABs feature the attractive properties of both aptamers and MBs, the design of a MAB must typically include an internal or external quencher (e.g., graphene oxide, carbon nanotubes, quantum dots).[5] These components can be expensive and add complication to the design. Inspired by the concept of quencher-free molecular beacons (QF-MBs),[6] we previously proposed a novel fluorescence sensor, namely a quencher-free molecular aptamer beacons (QF-MABs), that overcomes the limitations of traditional MABs.[7] The first reported DNA-based QF-MAB featured Szostak's 25-mer ATP binding sequence[8] in the loop region and formed hairpin structure with an elongated stem. As the name implies, QF-MABs do not contain a quencher, but they do incorporate one or more fluorophore units somewhere in the hairpin sequence. When a fluorophore is attached to the C5-position of a pyrimidine or the C8-position of a purine residue in a nucleoside, the hydrogen bonding of that residue is not disturbed. An aptamer typically binds to its target through an "induced fit" binding mechanism that results in a conformational change in the aptamer structure, such that a detectable signal is produced in QF-MAB.[9] Upon binding with ATP, QF-MAB undergo a conformational change to a folded-structure in the loop region, with a corresponding increase in the fluorescence intensity. Accordingly, this strategy can form a simple and cost-effective probing system.

We postulated that RNA aptamer sequences, as well as other aptamer sequences, could be modified to generate various fluorescence sensors through simple modification of the fluorescent nucleosides in QF-MAB systems. In addition, we suspected that it might be possible to overcome the limitations of sensitivity and selectivity found with DNA-based QF-MABs for ATP detection. We reported fluorescent biosensors prepared using their RNA aptamer as the receptor and a fluorescent ribonucleopeptide as the signal transducer.[10–14] The ATP-binding RNA aptamer An16 was

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applied to test whether a subtle change in the loop region could be exploited to selectively recognize ATP in RNA-based QF-MAB systems (Fig. 1B).[11] An16 possesses the 5′-GUAGUGG-UGUGUGUG-3′ consensus sequence and a relatively short (19-base) ATP binding sequence. This sequence has a small ATP-binding site and relative high affinity for ATP (K_d = 1.1 μM at 4 °C).[11] In addition, structural information for the ATP-binding receptor suggested that the ATP molecule formed 1:1 complex with An16. The adenine ring of ATP bound with uridine residues in the aptamer sequence to form a U:A:U triple, which stabilized the bent structure through A:U Hoogsteen base paring.[11] Combining the binding affinity of an An16 with the signal transduction arising from complexation of a RNA-based QF-MAB with ATP, it may be possible to establish sensitive and selective fluorescence probes for target species.

In this report, we demonstrate a fluorescent ATP aptasensor based on RNA-based QF-MAB systems featuring ATP-binding RNA aptamer sequences (Fig. 1A). The incorporation of fluorescent nucleoside into the structurally well-defined ATP-binding RNA aptamer led to the development of a new fluorescent probe for ATP detection. In the design of this RNA-based QF-MABs for ATP detection, the QF-MABs consisted of a stem/loop structure with the target-binding aptamer sequence in the loop region. In addition, fluorescent nucleosides capable of transducing the signal upon binding of the target could be inserted at the any position in the RNA-based QF-MAB sequences. According to this design, the ATP-binding RNA aptamer sequence was conserved after introducing the fluorescent nucleoside residues. In the absence of ATP, the fluorescence intensity of the RNA-based QF-MABs was low, due to quenching of the fluorescence of the nucleoside by neighboring bases and the single strand like structure in the loop region. Upon binding ATP, the RNA-based QF-MABs formed a secondary structure for the QF-MAB·ATP complex for which the fluorescence intensity was recovered. The change in fluorescence that reported ATP binding relied on the altered interactions between the fluorescent nucleoside and its neighboring bases due to structural changes. Therefore, this system features a single oligonucleotide strand bearing an environment-sensitive fluorescence nucleoside, without the need for additional quencher or any other additives to minimize the background signal and/or to transduce the signal upon ATP binding. Also, when the fluorescent nucleoside replaced the U moiety in the loop region, increased fluorescence appeared rapidly in the presence of ATP.

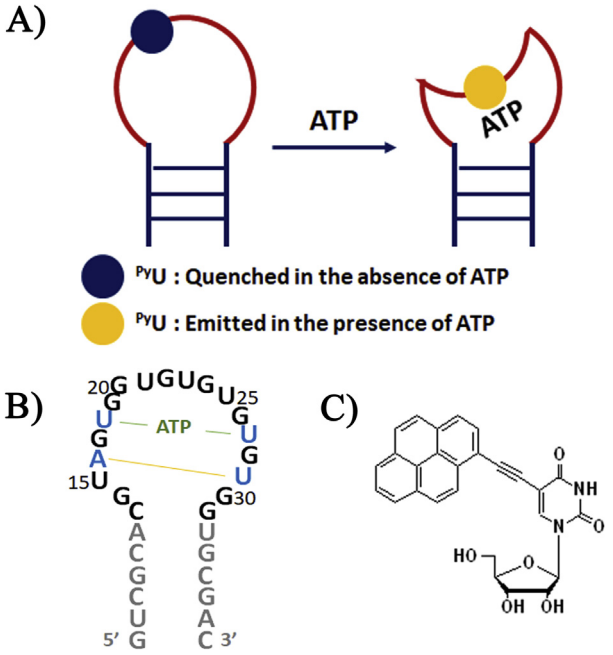

Fig. 1. (A) Schematic representation of a RNA-based quencher-free molecular aptamer beacon for ATP detection. (B) Sequence of RNA-based quencher-free molecular aptamer beacon for ATP detection; black/blue text: An16 (ATP aptamer sequence); blue text: ATP binding site; following the numbering system in Ref. 11. (C) Structure of the fluorescent nucleoside ^{Py}U.

As a proof of concept, we employed 5-(1-pyrenylethynyl)-uridine (^{Py}U) as a fluorescent nucleoside for the RNA-based QF-MABs (Fig. 1C).[15] In ^{Py}U, the pyrene group is separated from the uridine moiety by a rigid acetylene linkage. The fluorescence emission of pyrene displays is sensitive to its microenvironment.[16] Previous photophysical studies have revealed that the fluorescence properties of ^{Py}U are highly sensitive to structural changes arising from variations in its neighboring bases or base pairing. It has been reported that the fluorophore is efficiently quenched when in close proximity to an electron-donating guanosine unit; therefore, it is important to control the guanosine quenching effect when designing a QF-MABs.[6a] We synthesized ^{Py}U from 5-iodouridine and 1-ethynyl pyrene through Pd-catalyzed Sonogashira coupling, and then converted it into the corresponding phosphoramidite for oligonucleotide synthesis.[15] The modified nucleoside was incorporate into the designed oligonucleotide sequence to determine appropriate positions that led to enhanced signaling of the RNA-based QF-MABs. To investigate the effect of the position of modification of the RNA-based QF-MABs, we incorporate the fluorescent nucleoside residue at several positions in the loop region (Table 1)—namely at the ATP binding site or close to it, thereby maximizing the possibility of a change in fluorescence upon binding ATP (Fig. 1B; C13 to G31). The 6 bp stem region was appended at C13 and G31 to form a hairpin structure in the absence of ATP. We knew that U18 and U27 participate in the binding of ATP through the formation of U:A:U triplet with the adenine ring of ATP and additionally A16 and U29 hydrogen bonding in the presence of ATP (see the numbering system in Fig. 1B).[11] These fluorescent oligonucleotides were prepared through solid phase RNA synthesis and characterized using MALDI-TOF mass spectrometry (Table S1).

We investigated the change in fluorescence intensity of the RNA-based QF-MABs containing ^{Py}U upon addition of 10 mM ATP (Fig. 2). The pyrene fluorophore exhibits an environment-sensitive change in its fluorescence emission. When exposed to aqueous environments, strong fluorescence emissions are observed.[16] All of our ^{Py}U-modified ATP aptamer sequences exhibited increased fluorescence intensities in the presence of ATP than in its absence (i.e., they functioned as "turn-on" systems). First, we measured the fluorescence properties of ABU15, ABU18, and ABU21, where the

Table 1
Designed quencher-free molecular aptamer beacons for ATP detection.

Name	Sequence (5'→ 3')
ABU14	GUC GCA C^{Py}UU AGU GGU GUG UGU GUG GUG CGA C
ABU15	GUC GCA CG^{Py}U AGU GGU GUG UGU GUG GUG CGA C
ABU16	GUC GCA CGU ^{Py}UGU GGU GUG UGU GUG GUG CGA C
ABU17	GUC GCA CGU A^{Py}UU GGU GUG UGU GUG GUG CGA C
ABU18	GUC GCA CGU AG^{Py}U GGU GUG UGU GUG GUG CGA C
ABU20	GUC GCA CGU AGU G^{Py}UU GUG UGU GUG GUG CGA C
ABU21	GUC GCA CGU AGU GG^{Py}U GUG UGU GUG GUG CGA C
ABU22	GUC GCA CGU AGU GGU ^{Py}UUG UGU GUG GUG CGA C
ABU21NS	CAC GUA GUG G^{Py}UG UGU GUG UGG UG
ABS5	GC^{Py}U CCG UAG UGG UGU GUG UGU GGG AGC
ABS7	GAC UC^{Py}U CGU AGU GGU GUG UGU GUG GAG AGU C

The stem part is represented by underline.