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A target-triggered strand displacement reaction cycle: The design and application in adenosine triphosphate sensing

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ABSTRACT

A strand displacement reaction (SDR) system that runs solely on oligonucleotides has been developed for the amplification detection of adenosine triphosphate (ATP). It involves a target-induced SDR and an entropy-driven catalytic cycle of two SDRs with five oligonucleotides, denoted as *substrate*, *fuel*, *catalyst*, *C-1*, and *C-2*. *Catalyst*, released from the ATP aptamer–*catalyst* duplex by ATP molecule, catalyzes the SDRs to finally form the *substrate–fuel* duplex. All of the intermediates in the catalytic SDR processes have been identified by polyacrylamide gel electrophoresis (PAGE) analysis. The introduction of ATP into the SDR system will induce the ATP aptamer to form G-quadruplex conformation so as to release *catalyst* and trigger the SDR cycle. When the *substrate* and *C-2* oligonucleotides were labeled with a carboxyfluorescein (FAM) fluorophore and a 4-([4-(dimethylamino)phenyl]azo)benzoic acid (DABCYL) quencher, this SDR catalytic system exhibited a "turn-on" response for ATP. The condition for detecting ATP, such as Mg^{2+} concentration, has been optimized to afford a detection limit of 20 nM. This work provides an enzyme-free biosensing strategy and has potential application in aptamer-based biosensing.

The use of aptamers—single-stranded oligonucleotides with tailor-made sequences—as versatile receptors for high-affinity analyte binding has received a great deal of attention in the analytical and bioanalytical fields during recent years [1–4]. The sequence of aptamer is generally determined through the SELEX (systematic evolution of ligands by exponential enrichment) process [2,5]. The obtained sequences enable them to specifically bind to their targeted analytes with dissociation constants in the range of micromolar to picomolar. More than 100 aptamers have been established for the binding of organic dyes, amino acids, antibiotics, peptides, vitamins, proteins, and even whole cells or microorganisms [6,7]. Among these analytes is adenosine triphosphate (ATP),[1] the most important energy carrier in all living cells. It plays crucial roles in the regulation of cellular metabolism and has been widely used as an index in biomass determinations in clinical microbiological assays, food quality control, and environmental analyses [8,9]. Since the report of the DNA aptamer for ATP in 1995 [10], numerous ATP biosensors have been developed via coupling it to a variety of signal transduction mechanisms. These include fluorescence resonance energy transfer (FRET) [11–14], fluorescence/luminescence enhancement by dye intercalation [15], localized surface plasmon resonance (LSPR) responses from gold nanoparticles [16], and electrochemical detection [17,18].

Various new approaches have been explored to boost detection sensitivity of chemosensing and biosensing processes via the enhancement/amplification of sensing responses, including nanoparticle-based electrochemical methods [19–21], rolling cycle amplification [22,23], and some amplification strategies based on polymerase [24,25], nicking endonuclease [26], and exonuclease III [27,28]. To broaden the application range of the biosensing systems, the concept of enzyme-free amplification detection has been proposed [29–33]. The recently reported strand displacement reaction (SDR) of oligonucleotides has offered an attractive alternative for signal amplification in chemo-/biosensing. SDR occurs between a duplex containing a "toehold" and an oligonucleotide that is fully complementary with the longer sequence in the duplex. The incoming strand possesses nucleotide sequence that is complementary to this toehold as well as the rest of the longer oligonucleotide. Once paired with the toehold, the incoming strand displaces the outgoing strand via a strand exchange mechanism

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[1] *Abbreviations used:* ATP, adenosine triphosphate (adenosine 5′-triphosphate trisodium salt); FRET, fluorescence resonance energy transfer; SDR, strand displacement reaction; FAM, carboxyfluorescein; DABCYL, 4-([4-(dimethylamino)phenyl]azo)benzoic acid; TE buffer, Tris–EDTA buffer; UTP, uridine 5′-triphosphate trisodium salt; CTP, cytidine 5′-triphosphate disodium salt; GTP, guanosine 5′-triphosphate sodium salt; CD, circular dichroism; PAGE, polyacrylamide gel electrophoresis; RSD, relative standard deviation.

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goaded by the subsequent gain in entropy of the system [34–36]. By careful design of aptamers and the alignment of more than one SDR into catalytic reaction cycles, an oligonucleotide strand can "catalyze" the formation of multiple pairs of exchanged duplexes to realize signal amplification [37]. This entropy-driven, oligonucleotide-only catalytic cycle does not require any other reagents or enzymes to proceed and is particularly suitable for aptamer-based biosensing. Although such catalytic cycles have been employed to design amplification strategies for the detection of microRNA [38] and ATP [39] in horseradish peroxidase-assisted and polymerase-assisted systems, respectively, it has not been reported to conduct biosensing for small biomolecule detection in an oligonucleotide-only system so far.

In this work, we report an aptamer-based biosensing scheme for ATP using the catalytic SDR cycle proposed by Yurke and coworkers [37] with ATP molecules serving as the *initiator* (Fig. 1A). The *catalyst* strand is arrested by ATP aptamer until the addition of ATP. This *catalyst* can displace the *C-1* strand on a stable *S–C* complex (*substrate*, *C-1*, and *C-2*) and expose a domain on the *substrate* strand to facilitate the follow-up strand exchange with a *fuel* strand. Finally, the *S–F* duplex (*substrate–fuel*) is formed and the *catalyst* strand is released to catalyze the next cycle. No *S–F* duplex can be formed in the absence of *catalyst* because the corresponding region on *substrate* is occupied by *C-1* and *C-2*. This is an ATP-triggered cycle because the formation of G-quadruplex induced by ATP [10] releases the arrested *catalyst* strand that can catalyze the catalytic cycle. We have labeled the 3′ end of the *substrate* oligonucleotides with a carboxyfluorescein (FAM) fluorophore and labeled the 5′ end of the *C-3* oligonucleotides with a 4-([4-(dimethylamino)phenyl]azo)benzoic acid (DABCYL) quencher. As is shown in Fig. 1B, in the absence of ATP, the *catalyst* strand is arrested by ATP aptamer and the fluorescence is quenched due to the close proximity of FAM and DABCYL on the *substrate* and *C-2* strands. In the presence of ATP, the occurring SDR processes separate the quencher and fluorophore, which resumes the fluorescence. Because the ATP-released *catalyst* strand can be recycling in the catalytic cycle, the constructed biosensing strategy is, in essence, a signal amplification system.

Materials and methods

Chemicals and apparatus

All of the oligonucleotides used in this work were synthesized by Sangon Biotechnology (Shanghai, China). Their sequences are listed

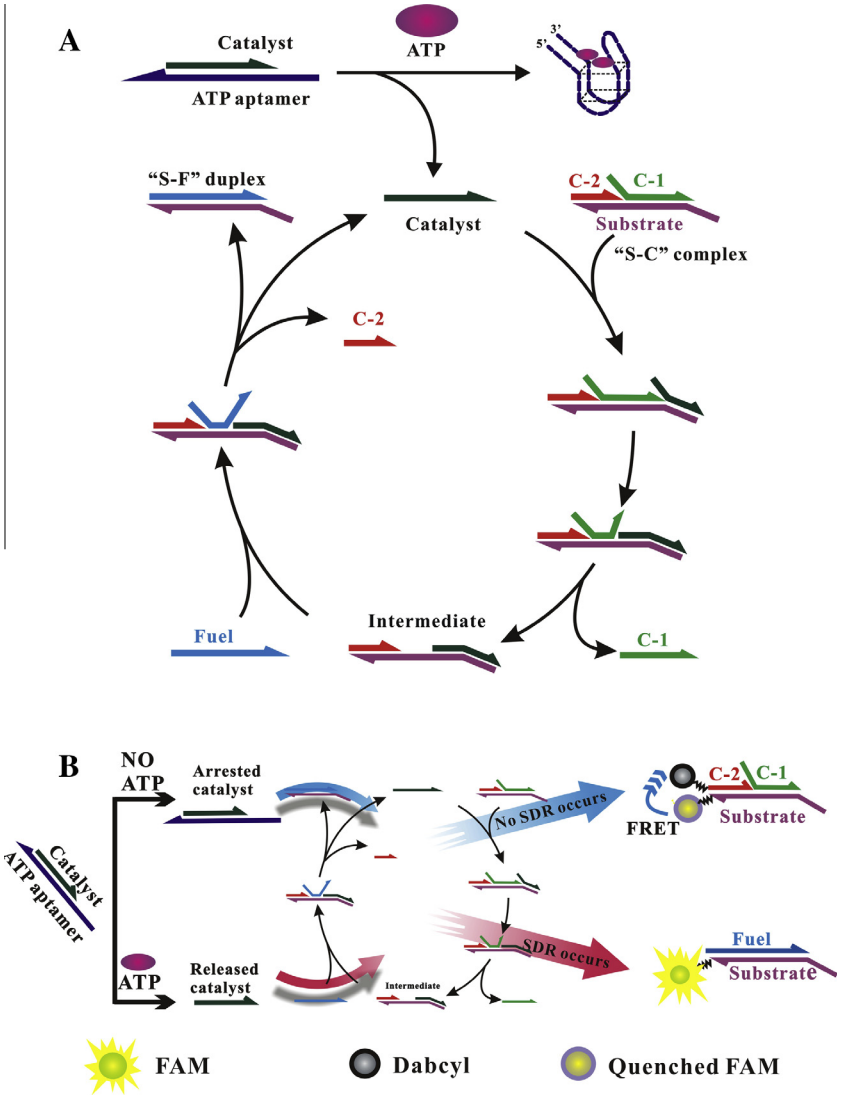

Fig.1. (A) Reaction mechanism of SDR-based catalytic cycle (arrows on DNA strands denote the direction from 5′ to 3′ terminals). (B) FRET detection scheme for ATP biosensing.