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Use of a small molecule as an initiator for interchain staudinger reaction: A new ATP sensing platform using product fluorescence

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ABSTRACT

We demonstrated that a small molecule induced interchain Staudinger reaction can be employed for highly selective detection of adenosine triphosphate (ATP), an important energy-storage biomolecule. A designed ATP split aptamer (A1) was first functionalized with a weakly fluorescent coumarin derivative due to an azide group (azido-coumarin). The second DNA strand (A2) was covalently linked with triphenylphosphine, which could selectively and efficiently reduce azido to amino group through the Staudinger reaction. The A2 was then hybridized with a half of another designed longer DNA strand (T1). The second half of T1 was a split aptamer and selectively recognized ATP with A1 to form a sandwich structure. The specific interaction between ATP and the aptamers drew the two functionalized DNA strands (A1 and A2) together to initiate the interchain Staudinger reduction at fmol-nmol concentration level, hence produced fluorescent 7-aminocoumarin which could be used as an indicator for the presence of trace ATP. The reaction process had a concentration dependent manner with ATP in a large concentration range. Such a strategy of interchain Staudinger reaction can be extended to construct biosensors for other small functional molecules on the basis of judiciously designed aptamers.

1. Introduction

Currently, template DNA fragments are often used to trigger an interchain organic reaction. As early in 1953, Watson and Crick first proposed the use of DNA as templates in organic synthesis by deducing the structure of a double strand DNA [1]. The concept of template-directed organic reaction has been applied on metal cations as templates in macrocyclization reactions [2]. Henceforward, molecular templates are used for the synthesis of carceplexes, catenanes, rotaxanes and so on [3,4]. In order to synthesize non-enzymatic oligonucleotide ligations and modifications, DNA-templated organic synthesis (DTS) has been explored for the evolution of generating synthetic small-molecule libraries of useful complexity [5–18]. Recently, DTS has also been used as a tool for detection of single nucleotide polymorphism of template DNA. Some studies in this field also focus on DNA-directed reactions between labeled oligonucleotides that contain quenched fluorophores or photosensitizers. The most extensively studied systems are nucleophilic ligation reactions, templated native chemical ligations and templated Staudinger reactions [8–13]. However, only using DTS as model for DNA detection limits its versatility and simplicity and thus narrows the range of application. Furthermore, the common Staudinger reactions have been reported involving in the reduction of individually designed profluorophores, thus broadening its application for bioactive small molecules.

ATP is an indispensable energy storage molecule in many biological processes such as in the regulation of cellular metabolism and biochemical pathways in physiological processes [19,20]. Many methods have been designed for the detection of ATP and other bio-related small molecules depend on different signal output mode [21–29]. Fluorescence labeling method are particularly encouraged, in part due to their high sensitivity, precise localization and feasibility of quantification [30,31]. Among these fluorescent assays, ATP aptasensors have been extensively utilized in ATP detection because of its stability and reusability [32–41]. To date, few reports describe the chemical reaction-

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triggered fluorogenic molecular probe for bio-related small molecule recognition. In considering that Staudinger reduction exhibited rapid kinetics and a high degree of bioorthogonality, we thus employed Staudinger reaction for the design of a new ATP aptasensor.

A1: 5′-N_3-coumarin-ATACCTGGGGGAGTATATAAT-3′
A2: 5′-TCGGATTCTA-TPP-3′
T1: 5′-ATATGCGGAGGAAGGTCATAGAATCCGA-3′

2. Experimental section

2.1. Materials and apparatus

All oligonucleotides were synthesized by TaKaRa Biotechnology Co. Ltd. (Dalian, China). All sequences were dissolved in highly pure water (sterile Minipore water, 18.3 MΩ•cm) as stock solutions, the concentrations of the solution were estimated by UV absorption using published sequence dependent absorption coefficients. Adenosine triphosphate (ATP), guanosine triphosphate (GTP), cytidine triphosphate (CTP) and uridine triphosphate (UTP) were purchased from Sigma-Aldrich and prepared using sterile water. All other chemical reagents were of analytical grade and were purchased from Fluka (Switzerland). All work solutions were prepared with 0.01 M sodium phosphate buffer (pH 7.0, 200 mM NaCl, 10 mM $MgCl_2$). UV–vis absorption spectra were recorded in 1 cm path length quartz cuvettes on a Hitachi U-3010 UV/vis spectrophotometer (Kyoto, Japan). The steady-state fluorescence spectra were obtained on a PTI QM4 Fluorescence System (Photo Technology International, Birmingham, NJ). Fluorescence spectra were collected from a 0.2 × 1.0 cm^2 quartz cuvette containing 500 µL of solution using a band width of 5 nm. The pH was measured by model 868 pH meter (Orion).

2.2. The designed DNA sequence and fluorescence monitor process

Synthesis of 7-azido-coumarin-4-acetic acid: 7-azidecoumarin-4-acetic acid was synthesized according to the reported synthetic procedure [42,43]. We conjugated two designed DNA with 7-azido-coumarin-4-acetic acid and triphenylphosphine, respectively, by using the succinimide coupling (EDC-NHS) method [44,45], and confirmed the ligation product by TOF-MASS and UV–Vis spectra. 50 nM of A2 and T1 were first mixed in a quartz cell containing 0.5 mL sodium phosphate buffer and were kept for 30 min at room temperature. 50 nM A1 and different concentration of ATP were then added to the solution and the Staudinger reaction process was monitored by recording the fluorescence of the product.

3. Results and discussion

Herein, we report a new kind of small molecular triggered Staudinger reduction between designed ATP split aptamers using adenosine triphosphate (ATP) as an initiator and demonstrate the application for ATP selective detection [46,47]. ATP has a wide distribution in organisms and cells which made this method more biocompatible. 7-azido-coumarin-4-acetic acid (Fig. S1) was first synthesized with weak fluorescence due to the azide group at the 7′ position. The ATP aptamer was then fabricated by cutting the 27-base aptamer of Szostak into 5′ fragment of 14-bases and 3′ fragment of 13-bases [48]. The binding ability of split aptamers and ATP is comparable with the reported monomeric aptamer by compared with the dissociation constant [49,50]. The design chosen specific recognition between aptamer and target as identify element on the basis of retain the DTS structure. So we proposed the strategy by introducing three designed DNA strands, A1, A2 and T1. The azido-coumarin and triphenylphosphine (TPP) were conjugated with 5′ fragment of the ATP split aptamer (A1) and the designed DNA sequence (A2), respectively. The structures of modified strands were confirmed by UV–Vis spectra and ESI-MS (Fig. S2 and Fig. S3). Moreover, the conjugation with DNA strands can enhance the water solubility of two reagents and also help the reaction taking place under lower concentration. T1 composes of 3′ fragment of ATP split aptamer and a strand moiety capable of completely hybridizing with A2. Unlike the DTS strategy, T1 in this system is not the identity of the template strand and used as the auxiliary strand for lowering background signal.

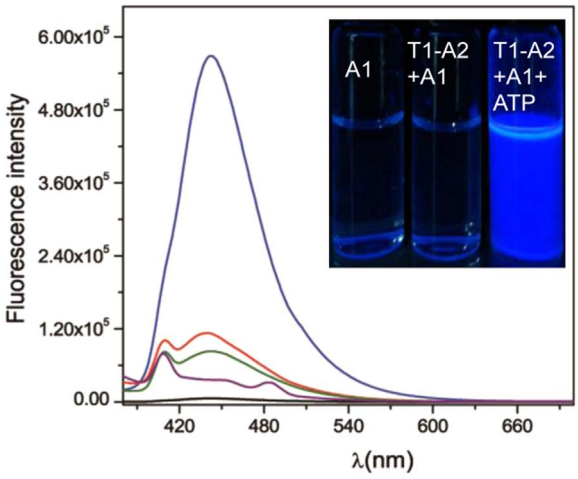

Fig. 1. Fluorescence spectra of different reaction systems in the phosphate buffer at room temperature. Black line: 50 nM N_3-Coumarin and 50 nM TPP in DMF, purple line: A1, green line: T1-A2 + A1, red line: A1 + A2, blue line: T1-A2 + A1 + ATP. Ex: 365 nm. The concentrations of the three strands are 50 nM. The inset shows the fluorescence image of 50 nM of each reaction system under a 365 nm UV lamp. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Actually, the background fluorescence could be greatly reduced by first hybridizing A2 with the auxiliary strand T1, as shown in Fig. 1. It can be seen that the simple mixture of A2 and A1 in aqueous solution produces fluorescence (Fig. 1, red curve). It is because that the electrostatic attraction and Van der Waals force of A1 and A2 still induce a small probability for the Staudinger reaction to occur. Differently, the mixture system of T1-A2 and A1 shows much weaker fluorescence than the simple mixture of A2 and A1, suggesting that sealing up A2 with a complementary DNA strand (T1) can limit random interactions between the two strands of A1 and A2 (Fig. 1, green curve). The background fluorescence of the system remains very low before adding ATP. Upon addition of ATP, however, the formation of ATP-directed sandwich structure between A1 and T1 drew the two conjugated DNA strands A1 and A2 in close proximity, which enhanced the effective molarity of the reactants and initiated the Staudinger reaction under nanomolar concentration (Scheme 1). Thus the fluorescence of the mixture was greatly enhanced due to the reduction of azido-coumarin, following a dose response manner (Fig. 1, blue curve).

Fig. 1 compares the fluorescence spectra of different systems of DNA strands at nanomolar concentration level (50 nM) in PBS solution before and after addition of ATP. It can be seen that the mixtures of modified strands A1, A2, and T1 show no fluorescence without addition of ATP. The addition of ATP rapidly induced brightly blue fluorescence of amino-coumarin, which was generated from the reduction of azido-coumarin by TPP, suggesting that the Staudinger reaction between azido and TPP moieties on the DNA strands took place. This result showed that ATP actually played the role as the initiator and increased the Staudinger reaction yield. Fig. 1 inset shows the fluorescence images of the system before and after the addition of ATP. It displays no fluorescence under irradiating with UV-light at 365 nm before ATP addition. Upon the addition of ATP, the system shows highly blue fluorescence, indicating the production of amino-coumarin.

In contrast, the Staudinger reaction between azido-coumarin and TPP without conjugation of DNA strands could not proceed well at