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Target-protecting dumbbell molecular probe against exonucleases digestion for sensitive detection of ATP and streptavidin

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ABSTRACT

In this work, a versatile dumbbell molecular (DM) probe was designed and employed in the sensitively homogeneous bioassay. In the presence of target molecule, the DM probe was protected from the digestion of exonucleases. Subsequently, the protected DM probe specifically bound to the intercalation dye and resulted in obvious fluorescence signal which was used to determine the target molecule in return. This design allows specific and versatile detection of diverse targets with easy operation and no sophisticated fluorescence labeling. Integrating the idea of target-protecting DM probe with adenosine triphosphate (ATP) involved ligation reaction, the DM probe with 5′-end phosphorylation was successfully constructed for ATP detection, and the limitation of detection was found to be 4.8 pM. Thanks to its excellent selectivity and sensitivity, this sensing strategy was used to detect ATP spiked in human serum as well as cellular ATP. Moreover, the proposed strategy was also applied in the visual detection of ATP in droplet-based microfluidic platform with satisfactory results. Similarly, combining the principle of target-protecting DM probe with streptavidin (SA)-biotin interaction, the DM probe with 3′-end biotinylation was developed for selective and sensitive SA determination, which demonstrated the robustness and versatility of this design.

1. Introduction

As an important small molecule in life activities, adenosine-5′-triphosphate (ATP) is a multifunctional nucleotide, which is not only a universal energy source, but also an extracellular signaling mediator and involved in many biological processes including DNA replication, membrane ion-channel pump, biosynthesis, hormonal and neuronal activities (Abraham et al., 1997; Newman et al., 1997; Szewczyk et al., 1998). It has also been used as an indicator of living organisms for cell viability and cell injury (Eguchi et al., 1997). Therefore, the highly sensitive and selective detection of ATP is essential for biochemical research, food quality control, environmental analysis, as well as clinical diagnosis. Many methods based on host-guest receptors, peptides, conjugated polymers, DNA/RNA aptamers, and ATP-dependent ligation reactions have been developed for ATP detection (Li et al., 2005; Lin et al., 2014; Lu et al., 2010, 2011; McCleskey et al., 2003; Mizukami et al., 2002; Zhou et al., 2011). Among these reported strategies, some of them exhibit only moderate sensitivity with detection limits for ATP in the micromolar or nanomolar range. In addition, even though some methods have shown desirable analytical performance, they usually require pre-labeling of a signal source, which needs considerable time-consumption and may suffer from higher cost.

The investigation of small molecule-protein interaction is of great significance in unveiling the mystery of cell development involving small molecules, as well as in the drug discovery and molecular diagnostics and therapeutics (Gao et al., 2004; Howitz et al., 2003; Overington et al., 2006; Stockwell, 2004). There are numerous analytical methods available for the assays of small molecules or their protein receptors, including capillary electrophoresis, affinity chromatography, surface plasmon resonance (SPR), fluorescence resonant energy transfer, and fluorescence anisotropy (Bachovchin et al., 2009; Drabovich et al., 2009; Goldman et al., 2005; Mano et al., 2006; Petrov et al., 2005; Wear et al., 2005). Typical of many molecule-protein interactions, the binding of biotin to streptavidin (SA) has been the subject of considerable fundamental and applied interest. To study the SA-biotin interaction, a novel method called terminal protection of small molecule-linked DNA was developed, which translated the small molecule-protein interaction into the detection of DNA (Wu et al., 2009). In view of the unique characteristics of DNA, such as specificity, stability, sequence coding, and the assists of various enzymes as well as enormous achievements of DNA sensors (Li et al., 2010; Zhang et al., 2013; Zhao et al., 2015), the strategy of terminal

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protection of small molecule-linked DNA opened a new window for studying the small molecule-protein interaction, and has been applied in numerous bioassays (Cao et al., 2012; He et al., 2013; Wang et al., 2013; Wu et al., 2011; Zhou et al., 2013).

Inspired by the idea of building the bridge of DNA detection for other molecules sensing, and based on our previous efforts in small molecule and protein assays (Chen et al., 2014a, 2016), a versatile dumbbell molecular (DM) probe was designed for multiplex and sensitive bioassays. In the presence of target analyte, the DM probe was protected from the degradation of exonucleases, consequently exhibiting obvious fluorescence response resulted from the protected DM probe bound to intercalation dye. United with the ATP dependent ligation reaction, the DM probe with 5′-end phosphorylation was constructed for sensitive detection of ATP which was chosen as target model of small molecule. Changing the same DM probe to the one with 3′-end biotinylation, the strategy of target-protecting DM probe against exonucleases digestion was also employed for the sensing of protein SA based on the specific SA-biotin interaction. In addition to the versatility and compatibility of the proposed strategy, the results indicated that both of the detections of ATP and SA were of high sensitivity and selectivity, as well as excellent resistance to matrix interferences. What's more, benefiting from the user-friendly control, this sensing strategy was also applied in the visual colorimetric analysis in droplet-based microfluidic platform.

2. Experimental

2.1. Materials and reagents

All oligonucleotide with different sequences were synthesized and HPLC purified by Sangon Biotechnology Co., Ltd (Shanghai, China). The sequences of the oligonucleotide used in this work are listed in Table S1 (Supplementary information). Exonuclease I (Exo I), Exonuclease III (Exo III), and Shrimp alkaline phosphatase (SAP) were purchased from the Takara Biotechnology Co., Ltd. (Dalian, China). T4 DNA ligase was purchased from Thermo Fisher Scientific. SYBR Green I (SG I) (10,000 ×), Streptavidin (SA), adenosine triphosphate (ATP) and its analogues were obtained from Sigma-Aldrich. Human serum sample was supplied by the Zhongnan Hospital of Wuhan University (Wuhan, China). Cell culture dishes were was obtained from NEST Biotechnology (Beijing, China). Culture medium and fetal bovine serum (FBS) were purchased from Gibco BRL (Grand Island, NY, USA). The silicon wafer was purchased from Institute of Microelectronics of Chinese Academy of Sciences. The poly(dimethylsiloxane) (PDMS) and AZ 50XT photoresist were obtained from RTV615 GE Toshiba Silicones Co., Ltd. and AZ Electronic Materials USA Corp., respectively. All chemical reagents were of analytical grade and used without further purification. All solutions were prepared with ultrapure water (18.25 MΩ cm) from a Millipore system.

2.2. Procedures for ATP assay

In a typical procedure, first, the DM probe 1 was diluted with 25 mM Tris–HCl buffer (100 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, pH = 7.6) and denatured at 95 °C for 10 min followed with a slow annealing treatment for 1 h before use. Then the DM probe 1 was sealed by adding 4 μL of T4 DNA ligase (5 U/μL), 5 μL different concentrations of ATP and 100 μL of Tris–HCl buffer into the 10 μL of 0.1 μM DM probe 1 solution and allowing the ATP-triggered ligation reaction at room temperature for 30 min. After that, 4 μL of Exo I (5 U/μL) and 10 μL of Exo III (20 U/μL) were added into the mixture solution to induce the digestion for 60 min. After that, 2 μL of SG I (50 ×) was added into the solution. Finally, the solution was diluted to 400 μL with Tris–HCl buffer and the fluorescence spectrum was obtained with a RF-5301PC spectrophotometer (Shimadzu, Japan) equipped with a 150 W xenon lamp (Ushio Inc, Japan).

2.3. Agarose gel electrophoresis

After the ligation and digestion reactions mentioned above, the mixture was stained with 100 × SG I (1 μL). Three percent of agarose gel was prepared using 1 × TAE buffer (40 mM Tris-AcOH, 2 mM Na_2EDTA, pH = 8.5). The electrophoresis was carried out at 100 V for approximately 40 min in 1 × TAE buffer. Then the gel was imaged by a ChemiDoc XRD system (Bio-Rad).

2.4. Cellular ATP assay

HepG2 cells were maintained in Dulbecco's modified eagle's medium (DMEM) supplemented with 5% fetal bovine serum (FBS), 10 mM Hepes, antibiotics (50 U/mL pencillin and 50 μg/mL streptomycin), and 2 mM glutamine at 37 °C under 5% CO_2 atmosphere. The collected cells were centrifuged at 3000 rpm for 3 min at 4 °C and washed three times by PBS buffer (20 mM phosphate, 100 mM NaCl, and 5 mM KCl, pH = 7.4) and then suspended in 25 mM Tris–HCl buffer (100 mM $NaNO_3$, 6 mM magnesium acetate, pH = 7.6). Cell lysis was performed by repeated cycles of freezing and thawing, and then the lysed cells were ready for ATP assays. For control experiment, the above HepG2 cell lysate (approximately 20,000 cells/mL, 400 μL) was treated with 10 units SAP at 37 °C for 20 min to remove the ATP.

2.5. Visual detection of ATP in droplet platform

The droplet microchip designed and fabricated according to the previous protocol (Chen et al., 2015b). For ATP assay, the DM probe 1 was sealed by adding 8 μL of T4 DNA ligase (5 U/μL), 5 μL different concentrations of ATP and 17 μL of Tris–HCl buffer into the 10 μL of 5 μM DM probe 1 solution and allowing the ATP-triggered ligation reaction at room temperature for 30 min. After that, 5 μL of Exo I (5 U/μL) and 5 μL of Exo III (200 U/μL) were added into the mixture solution to induce the digestion for 60 min. After that, the mixture solution was delivered from the sample container into the microchip for droplets generation by using the technique previously reported (Chen et al., 2015a). Finally, the droplets containing the protected DM probe 1 were mixed with the SG I reagent through the droplet dosing strategy. The fluorescence images were recorded by an inverted fluorescence microscope (Axio Observer.A1, Zeiss, Germany) in conjunction with a light source system (excitation filter was set as 490 nm) and a Spot RT3 charge-coupled device (CCD, Diagnostic Instruments, Inc., USA). The fluorescence intensities of the droplets were obtained by Image-Pro Plus software.

2.6. Procedures for SA detection

First, the DM probe 2 was diluted with 25 mM Tris–HCl buffer (100 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, pH = 7.6). 10 μL of 0.2 μM DM probe 2 was mixed with 5 μL different concentrations of SA and followed by adding 100 μL Tris–HCl buffer. The solution was incubated in room temperature for 30 min Then, 4 μL of Exo I (5 U/μL) and 10 μL of Exo III (20 U/μL) were added into the mixture solution to induce the digestion for 60 min. After the digestion reaction, 2 μL of SG I (50 ×) was added into the solution. Finally, the solution was diluted to 400 μL with Tris–HCl buffer and the fluorescence intensity was measured.