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A fluorescent biosensing platform based on the polydopamine nanospheres intergrating with Exonuclease III-assisted target recycling amplification

Weibing Qiang, Xi Wang, Wei Li, Xiang Chen, Hui Li, Danke Xu *

State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, 22 Hankou Road, Nanjing 210093, China

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ABSTRACT

Rapid, cost-effective, sensitive and specific analysis of biomolecules is important in the modern healthcare system. Here, a fluorescent biosensing platform based on the polydopamine nanospheres (PDANS) intergrating with Exonuclease III (Exo III) was developed. Due to the interaction between the ssDNA and the PDANS, the fluorescence of 6-carboxyfluorescein (FAM) labelled in the probe would been quenched by PDANS through FRET. While, in the present of the target DNA, the probe DNA would hybridize with the target DNA to form the double-strand DNA complex. Thus, Exo III could catalyze the stepwise removal of mononucleotides from 3′-terminus in the probe DNA, releasing the target DNA. As the FAM was released from the probe DNA, the fluorescence would no longer been quenched, led to the signal on. As one target DNA molecule could undergo a number of cycles to trigger the degradation of abundant probe DNA, Exo III-assisted target recycling would led to the amplification of the signal. The detection limit for DNA was 5 pM, which was 20 times lower than that without Exo III. And the assay time was largely shortened due to the faster signal recovery kinetics. What is more, this target recycling strategy was also applied to conduct an aptamer-based biosensing platform. The fluorescence intensity was also enhanced for the assay of adenosine triphosphate (ATP). For the Exo III-assisted target recycling amplification, DNA and ATP were fast detected with high sensitivity and selectivity. This work provides opportunities to develop simple, rapid, economical, and sensitive biosensing platforms for biomedical diagnostics.

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1. Introduction

For the inherent advantages of high sensitivity, operational convenience, and in situ imaging properties, molecular beacons (MBs) consisting of a FRET pair, have been elaborately designed and employed for the analysis of biomolecules in the fields of drug discovery, environmental monitoring, food safety, clinical diagnostics and treatment (Huang et al., 2014b; Kolpashchikov, 2010). Over the past few years, many nanomaterial-based, MBs-like fluorescent biosensors have been developed using nanomaterials as quenchers, such as gold nanoparticles (AuNPs) (Dubertret et al., 2001; Maxwell et al., 2002), carbon nanotubes (CNTs) (Yang et al., 2008) and graphene oxide (GO) (Lu et al., 2009; Swathi and Sebastian, 2008). These nanoquenchers are available to eliminate the selection issue of a fluorophore-quencher pair, due to their ability to quench fluorophores with different emission frequencies, and improve the signal-to-noise ratio of DNA probes. And these nanoquenchers have been successfully used for the detection of nucleic acids, proteins, metal ions, small molecules and enzyme activities (He et al., 2013; Jang et al., 2013; Li et al., 2015; Tu et al., 2013; Wang et al., 2011; Wen et al., 2011). The outstanding fluorescence quenching ability of polydopamine (PDA) have been reported in our previous work (Qiang et al., 2014b). And a sensing platform based on PDA was also developed for the detection of DNA and proteins with the best sensitivity among the nanoquenchers based biosensors (Qiang et al., 2014b). For the excellent biocompatibility and biodegradability of PDA, and the facile, low-cost preparation method, it has potentials to be developed into simple, rapid, and economical biosensors for molecular diagnostic.

To improve the detection sensitivity, various target and signal amplification techniques have been developed. For target amplification, a classic example is the polymerase chain reaction (PCR) (Botes et al., 2013; Kristensen and Hansen, 2009), which is a gold standard in DNA detection in terms of LOD. However, it requires an expensive thermal cycler, which limits its application in point-of-care settings. As thermal cycler-free alternatives to PCR, isothermal

* Corresponding author. Fax: +86 25 83595835.
E-mail address: xudanke@nju.edu.cn (D. Xu).

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target amplification methods have emerged recently, such as helicase-dependent amplification (HDA) (Andresen et al., 2009; Jeong et al., 2009), loop-mediated isothermal amplification (LAMP) (Han, 2013; Njiru, 2012), and recombinase polymerase amplification (RPA) (Santiago-Felipe et al., 2014; Xia et al., 2014). However, all target amplification strategies are sensitive to cross-contaminations, which produce false-positives. Therefore, require trained personnel and clean laboratory space. Another technique is to use chemical approaches to amplify the signal triggered by the presence of a target rather than the target itself. The examples of signal amplification approaches are branched DNA (bDNA) technology (Baumeister et al., 2012; Tsongalis, 2006), hybridization chain reaction (HCR) (Ge et al., 2014; Huang et al., 2014a; Li et al., 2014), enzyme-assisted target recycling (EATR) (Gerasimova and Kolpashchikov, 2014; Xue et al., 2012). In EATR, the probe–target complex is specifically recognized by an enzyme (usually nuclease), and only the probe strand is cleaved. The cleaved probe generates a detectable signal. The fragments of the cleaved probe have lower affinity to the target, compare to the intact probe. This results in the target release for binding to another molecule of the probe and triggering its cleavage. Cycling of recognition and cleavage enables amplification of the signal. The linear amplification of EATR reduces the false-positive results created by exponential amplification techniques. In addition, most of the EATR assays are isothermal process.

Among the enzymes in EATR, Exonuclease III (Exo III) seems to be more commonly used (Gerasimova and Kolpashchikov, 2014). As an enzyme in the 3′–5′ exonuclease family, Exo III exhibits 3′–5′ exodeoxyribonuclease activity. Exo III shows many unique properties: it catalyzes stepwise removal of mononucleotides in the direction from the 3′ to 5′ terminus of duplex DNA, thus enabling selective hydrolysis of one single-stranded DNA in duplex DNA; DNA strands with blunt ends, recessed 3′-ends or nicks are preferred for Exo III cleavage, while single-stranded DNA and the 3′-protruding end (that are at least 4 bases long) of double-stranded DNA are resistant to cleavage; Exo III does not require a specific sequence to function (Mol et al., 1995). These properties have evoked the use of Exo III in developing a variety of amplification platforms for homogeneous detection of nucleic acids, proteins and other targets (Hu et al., 2014; Liu et al., 2014a, 2014b; Wang et al., 2014; Zhang et al., 2013).

To our knowledge, the employment of those amplification strategies in the PDA based sensing platform to amplify the signal has not been reported yet. Here, a fluorescent biosensing platform based on the polydopamine nanospheres (PDANS) intergrating with Exo III-assisted target recycling amplification was developed. Due to the interaction between the ssDNA and the PDANS, the probe DNA would be adsorbed on the surface of the PDANS, thus the fluorescence of the FAM would been quenched through FRET. While, for the recognition of the target DNA, there would be a blunt 3′-terminus in the probe DNA, Exo III could then catalyze the stepwise removal of mononucleotides from this terminus, releasing the target DNA. And, the fluorescence of the FAM released from the probe would no longer been quenched by the PDANS, led to the signal on. As one target DNA molecule could undergo a number of cycles to trigger the degradation of abundant probe DNA, Exo III-assisted target recycling would led to the amplification of the signal for the assay of DNA. For the high specificity and affinity of aptamer, the sensing platform was also applied for the detection of ATP. DNA and ATP were fast detected with high sensitivity and selectivity, and the biosensing platform holds great potentialities in clinical applications.

2. Material and methods

2.1. Materials and reagents

Dopamine hydrochloride, adenosine triphosphate (ATP), guanosine triphosphate (GTP), cytidine triphosphate (CTP), and uridine triphosphate (UTP) were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). Exonuclease III (Exo III) was obtained from Thermo Scientific (USA). All other reagents were of analytical reagent grade and used without further purification. 20 mM Tris–HCl buffer (pH 7.4, containing 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$ and 1 mM $CaCl_2$) was used in this experiment. DNA sequences were synthesized by Sangon, and the sequences were as following (the mismatched bases are underlined):

P1: 5′-FAM-CCA ACC ACA CCA ACC-3′,
T1: 5′-GGT TGG TGT GGT TGG ACG T-3′,
T2: 5′-GGT TGG T<u>C</u>T GGT TGG ACG T-3′,
T3: 5′-GGT <u>A</u>GG T<u>C</u>T GG<u>A</u> TGG ACG T-3′,
P2: 5′-FAM-ACC TGG GGG AGT ATT GCG GAG GAA GGT-3′.

2.2. Apparatus

Scanning electron microscopy (SEM) images were recorded using an S-4800 scanning electron microscope (Hitachi, Japan). IR spectra were determined using a Nicolet 6700 FT-IR Spectrometer. A UV-3600 Spectrophotometer (Shimadzu) was used to obtain UV–Visible spectra. The fluorescence measurements were performed at room temperature on a Shimadzu RF-5301PC Fluorophotometer.

2.3. Preparation of PDANS

PDANS was synthesized according to our previous report with some modifications (Qiang et al., 2014b). Briefly, 100 mg dopamine hydrochloride was added to a mixture of 100 mL Tris-buffer (10 mM) and 40 mL isopropyl alcohol with stirring. After stirring for 72 h, PDANS was obtained. The suspension was centrifuged and washed/resuspended with water several times. The precipitate was dried for the following experiments.

2.4. Assay of DNA with Exo III-assisted target recycling

100 U Exo III was added to the mixture of fluorescent probe DNA P1 and different concentration of target DNA T1 in Tris–HCl buffer. After the mixture was incubated at 37 °C for 30 min and another 5 min at 75 °C to terminate the Exo III reaction, PDANS was added in the reaction mixture for 10 min before the fluorescent detection. The fluorescence emission spectra were recorded from 500 to 650 nm at an excitation wavelength of 470 nm, and the fluorescence intensity at 518 nm was used for quantitative analysis.

2.5. Assay of ATP with Exo III-assisted target recycling

The assay of ATP was similarly with that of DNA. The mixture of Exo III, fluorescent probe P2 and different concentration of target ATP was incubated at 37 °C for 30 min and then 75 °C for 5 min. PDANS was mixed with the reaction mixture at room temperature, and 10 min later, the emission spectra were recorded.

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