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Detection of biotin-streptavidin interactions based on poly(thymine)-templated copper nanoparticles coupled with Exo III-aided DNA recycling amplification

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ABSTRACT

The recognition and analysis of small molecule–protein interactions plays an important role in chemical genetics, clinical diagnostics, and drug development. However, various established highly sensitive fluorescence strategies still involve the use of expensive fluorescence dye-labelled nucleic acids. Herein, we constructed a highly sensitive and label-free fluorescent assay for small molecule–protein interactions on the basis of exonuclease III (Exo III)-assisted signal amplification and poly(thymine)-templated fluorescent copper nanoparticles (CuNPs) as signal probes. Biotin–streptavidin system was taken as a model. It was designed that biotinylated double-strand DNA served as the binding probe. Biotin was labelled at the 3′-end of the trigger strand. One kind of stem-loop DNA, which consisted of the thymine rich sequence and the trigger DNA recognition domain, was also designed. Once streptavidin bind to the binding probe, with the help of Exo III digestion, the trigger strand was able to reach the cycle for multiple poly(thymine) sequences generation. The produced poly(thymine) sequences can be used as a template for the formation of CuNPs. Comparing with no Exo III-aided DNA recycling amplification, significantly improved signals for the quantitative determination of streptavidin were obtained.

1. Introduction

Recently, there has been an increasing interest in the synthesis and application of ultra-small fluorescent nanoparticles including several noble metal nanoclusters, such as Au, Ag, and Pt nanoclusters, attributed to their unique physical, electrical, and optical properties [1]. The scaffolds for fluorescent metal nanoparticles synthesis include dendrimers, polymers, DNA, proteins and peptides etc [2–8]. In particular, DNA has attracted special research interest because of its unique nanosized structure, excellent programmable properties, and great affinity for some noble-metal ions [9–12]. Through the binding of metal ions on the DNA, and subsequent chemical reduction of the DNA-complexed metal ions, DNA-templated fluorescent metal nanoparticles were prepared [13]. They show advantages of easy synthesis, low toxicity, good biocompatibility, tunable fluorescence emission, high photo-stability. These properties make them suitable for biological application. So far, DNA-templated silver [14], palladium [15], platinum [16], copper [17], gold [18], nickel [19] and cobalt [20] have been obtained. Among them, copper nanoparticles (CuNPs) attract extensive research interest due to their advantages of easy preparation, excellent fluorescence and low toxicity. Rotaru et al. [9] found that random (double-stranded DNA) dsDNA could act as an efficient template for the formation of CuNPs, whereas random (single-stranded DNA) ss-DNA or triplex DNA did not support the formation of CuNPs. The dsDNA templated CuNPs act as efficient fluorescent probes for metal ions, small molecules, protein, and enzyme assays in further studies [21–25]. Lately, Qing et al. found that single-stranded poly(thymine) (poly T) could also be employed as a promising template for the formation of fluorescent CuNPs [17]. The CuNPs formation templated by poly T was due to binding interactions between thymine and Cu^{2+}, and the thymine-complexed Cu^{2+} ions were chemically reduced to Cu^{0} by the reducing agent. Additionally, the size and the fluorescence intensity of the CuNPs could be simply regulated by the length of the poly T. Attractively, compared with other DNA-templated fluorescent metal nanoparticles, such as dsDNA-templated CuNPs, silver nanoclusters, and gold nanoclusters, the synthesis of poly T-templated CuNPs was much faster and more convenient. It only takes several minutes

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to produce high quality poly T-templated CuNPs under ambient conditions. These properties of CuNPs form a basis for constructing novel biosensing platform for chemical/biological analysis [25–29].

The investigation of small molecule–protein receptor interaction is of great important in anti-cancer therapies, tumor-specific drug delivery, therapeutic agents, and molecular diagnostics [30–33]. Numerous analytical methods, such as affinity chromatography [34], kinetic capillary electrophoresis [35,36], field effect transistors [37], and protein-fragment complementation assay [38,39] have been established for detecting the small molecule–protein interactions. However, these techniques generally require sophisticated instruments specific labelling or immobilizing reagents, cumbersome assay procedures, and still show limited sensitivities. To get rid of the drawbacks of the existing methods, recently, Jiang's group has reported on a terminal protection strategy that involves the binding of small molecule-linked DNA to target proteins to protect the DNA from exonuclease-mediated digestion, which is not possible with only small molecule-linked DNA [40,41]. This finding forms a basis for constructing a versatile toll for electrochemical and fluorescent detection of small molecule-protein interaction. Inspired by the contribution of Jiang's group, many researchers have developed different analytical strategies for small molecule–protein interaction assay [40–45]. In order to improve the sensitivity of detection, some amplified methods were developed. Zhou et al. developed an ultrasensitive method based on molecular beacon and exonuclease III (Exo III)-aided DNA recycling method [46]. Their "turn-on" strategy produced the linear response of 8.3 fM to 83.3 pM, with a LOD of 0.8 fM. Ou et al. developed another Exo III-aided DNA recycling method for small molecule–protein interaction assay by using TaqMan probes as the reporter probes [47]. For folate receptor, they obtain a detection limit of 0.8 pM. T7 exonuclease-aided DNA amplification method was also been used for small molecule–protein interactions analysis [48]. Although promising, these strategies may suffer from drawbacks which still involve the use of expensive double fluorescence dye-labelled nucleic acids. This will certainly increase the cost and complexity of the assays. Therefore, it is highly desirable to develop facile label-free methods for small molecule–protein interaction assay.

Herein, by using poly T-templated CuNPs as signal reporter, combing with Exo III-aided DNA recycling method, we developed a novel label-free strategy for small molecule–protein interaction assay based on terminal protection strategy. Biotin–streptavidin system was taken as a model. Compared to the traditional amplifying methods, the proposed strategy is low costly and convenient, exhibiting high analytical performance and showing great potential in the analysis of other small molecule–protein interactions.

2. Experimental

2.1. Reagents

All oligonucleotides with different sequences were synthesized and purified with HPLC by Sangon Biotechnology Co., Ltd (Shanghai, China). The sequences of the oligonucleotide used in this work are as follows:

Trigger strand: 5′-TAGTGACATTTTTTTTTTTTTTTCGCGC-biotin-3′

Antisense strand: 5′-GCGCGAAAAAAAAAAAAAAATGTCACTA-3′

Stem-loop DNA: 5′-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTAAAA-AAAAAAAAAAATGTCACTA-3′

T30: 5′-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTT −3′

Exonuclease III was purchased from New England Biolabs. Sodium ascorbate was purchased from Sigma-Aldrich, Inc. (Shanghai, China). Streptavidin was purchased from Amresco (USA). Copper sulfate ($CuSO_4$) was of analytical grade. The reaction buffer solutions employed in this work were 10 mM Tris-HCl (pH 7.4), 10 mM $MgCl_2$, and 100 mM NaCl. The MOPS buffer (10 mM MOPS, 150 mM NaCl, pH 7.6) was used for the formation of fluorescent CuNPs. All Milli-Q purified water was used to prepare all the solutions.

2.2. Apparatus

Fluorescent emission spectra were performed on Varian cary eclipse fluorescence spectrophotometer, Varian Medical Systems, Inc. (Palo Alto, American). The sample cell is a 700-μL quartz cuvette. The fluorescence emission spectra were collected from 525 nm to 650 nm at room temperature with a 340 nm excitation wavelength. The slits for excitation and emission were set at 5 nm, 10 nm respectively. The fitting of the experimental data was accomplished using the software Origin 8.0.

2.3. Performance of biotin-SA interaction assay

For quantitative measurement of biotin–SA interaction, a fixed concentration of binding probe DNA (250 nM) was treated with different concentrations of SA (0 nM, 0.0692 nM, 0.346 nM, 0.692 nM, 1.384 nM, 1.73 nM, 3.46 nM, 6.92 nM, 13.48 nM, 17.3 nM, 34.6 nM, 69.2 nM, 138.4 nM and 173 nM) and shaken gently for 20 min at 37 °C. After that, 10 μL stem-loop DNA (10 μM) (final concentration: 1 μM) and 10 U Exo III was added to the solution. Then Tris-HCl buffer was added to the mixture to make the volume of the reaction solution to be 50 μL. At this condition, the reaction concentration of Exo III was 0.2 U/μL. The mixed solution was incubated for 60 min at 37 °C. 40 μL of MOPS buffer (10 mM MOPS, 150 mM NaCl, pH 7.6), 5 μL of sodium ascorbate (100 mM), and 5 μL of $CuSO_4$ (10 mM) were added into the solution to give a final volume of 100 μL and was allowed to react for 5 min at room temperature (25 °C), followed by the fluorescence measurement with an excitation wavelength of 340 nm.

3. Results and discussion

3.1. Design strategy

Due to its simplicity, high efficiency, rapidity, and hypotoxicity, the poly T-templated fluorescent CuNPs hold great potential for signal transducing as an in situ synthetic nanoprobe. By taking advantage of the exceptional properties of poly T-templated fluorescent CuNPs, a novel, highly sensitive and label-free method for small molecule–protein interaction assay based on poly T-templated CuNPs coupled with Exo III-assisted signal amplification has been proposed in this work. Fig. 1 illustrates the sensing strategy. Biotin–streptavidin system was taken as a model. As illustrated in Fig. 1, we designed an unlabelled stem-loop DNA. The stem-loop DNA contains two domains, which are identified as I and II according to their different functions. Region I (red) is the thymine rich sequence (T30), which could effectively template the formation of fluorescent CuNPs according to the literature [25]. Region II (blue) is the trigger-strand DNA recognition domain. In the stem-loop DNA, region I (T30) is partially caged in the duplex structure of the stem by hybridization with region II. As a result, the T-rich segment (region I) is prohibited to act as the template for the formation of CuNPs. To avoid the degradation of the stem-loop DNA by Exo III, a 8-nt single-stranded sequence protruded from the 3′-end of the stem-loop DNA. The formation of the stem-loop structure is thermodynamically favorable with a ΔG of −15.6 kcal/mol at room temperature that is calculated by DINAMelt server (http://unafold.rna.albany.edu/?q=DINAMelt). It is also stable at 37 °C ($\Delta G = -10.0$ kcal/mol), which is the optimal working