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A label-free fluorometric assay for actin detection based on enzyme-responsive DNA-templated copper nanoparticles

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ABSTRACT

Herein, we develop a fluorescent strategy for label-free detection of actin by the *in situ* formation of double-stranded DNA (dsDNA) templated copper nanoparticles (CuNPs) and the digestion capability of deoxyribonuclease I (DNase I). In this design, the introduction of actin can effectively hinder the enzymic digestion owing to the exceptional target-mediated conformational structure between actin and DNase I. Consequently, the remaining adenine-thymine-rich dsDNA can act as an efficient template for fluorescent CuNPs (λ_{ex} = 340 nm, λ_{em} = 585 nm) with a Mega-Stokes shifting (245 nm). This novel fluorescent strategy exhibits several advantages such as low-cost and environmental-friendly, because it does not require the toxic organic dyes and laborious procedures. Under optimized experimental conditions, this method realizes a turn-on selective and sensitive response for actin with a detection limit of 0.12 μg mL^{-1} and a detection capability in complex biological media with satisfactory results.

1. Introduction

Actin, the major structural component of the dynamic microfilament cytoskeleton system, exists in almost all eukaryotic cells and plays vital roles in muscle and cell motility, such as cytokinesis, membrane ruffling, cell locomotion, pinocytosis, and maintenance of cell shape [1]. Owing to their biological significance, actin has been a focus of intensive studies ever since its discovery [2]. Therefore, it is essential for the development of sensitive and selective methods for the detection of actin in the fields of molecular biology, genetics, and bio-medicine. A number of traditional tools for analysis of actin have been investigated, including polyacrylamide gel electrophoresis (PAGE), enzyme-linked immunosorbent assay (ELISA), and isotope labeling, which are relatively time-consuming and resource-intensive methods [3–5]. Hence, it will be desirable to develop fluorescent methods for actin detection, because fluorescent strategies are able to partly overcome limitations of conventional assays and provide promising advantages, such as high sensitivity, rapid analysis, and little damage to sample [6].

A number of enzymatic reactions show specific dependence on significant biomolecules, which, in turn, offer an efficient fluorescent method for developing selective sensing platforms for these biomolecules. For example, by taking advantage of the ligation-triggered cascade strategy, adenosine triphosphate (ATP) or nicotinamide adenine dinucleotide (NAD^+) can be distinguished from their analogs with an excellent sensitivity [7]. Besides, nucleases that preferentially attack the phosphodiester bonds to produce DNA fragments, such as exonuclease (Exo) I and Exo III, exhibit target-induced structure-sensitive digestion in fluorescent sensing systems [8,9]. Yet, the majority of current enzymatic methods require expensive fluorescent labeling process and complex chemical modification [10]. In addition, these fluorescent strategies frequently suffer from inherent shortages, including relatively high toxicity, poor photo-stability, low emission intensity, and small Stokes shifting [11]. Therefore, in order to improve the potential applicability, “green” nano-dyes as alternatives will be of great benefit to enzymatic sensing systems.

Compared to organic dyes and quantum dots, DNA-stabilized metal nanoparticles that exhibits excellent optical properties, facile surface modification and good bio-compatibility have been developed as a new class of fluorophores in the past years. Recently, fluorescent DNA-templated copper nanoparticles (DNA-CuNPs) as novel indicators have received emerging attention by using specific double-stranded DNA (dsDNA) or poly thymine as efficient templates [12,13]. Attractively, their fluorescent emission signals (λ_{ex} = 340 nm, λ_{em} = 570–615 nm)

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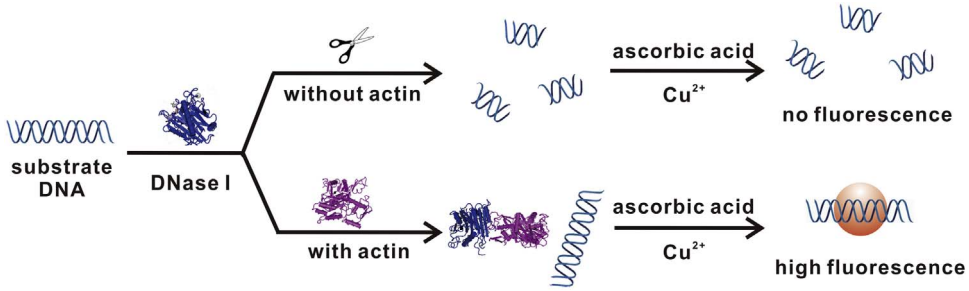

Scheme 1. Schematic illustration of the proposed fluorescent strategy for actin detection.

with a Mega-Stokes shifting can be simply obtained in several minutes upon the addition of copper ions and ascorbate at the room temperature. It has been accepted that both the length of DNA and the selected sequence can act as templates for the controllable *in situ* synthesis of fluorescent CuNPs, which improves the deep integration of fluorescence analysis and nanotechnology in life science [14]. For instance, He and co-workers recently developed a label-free and highly sensitive fluorescent strategy for the turn-on determination of ATP based on the formation of CuNPs within adenine-thymine (AT) rich regions of dsDNA templates [15]. Yet, there is still at an early stage for the biochemical application of fluorescent CuNPs [16].

Herein, DNA-CuNPs are employed as a fluorescent indicator for turn-on and label-free detection of actin by using the specific digestion capability of deoxyribonuclease I (DNase I). This strategy is derived from our previous studies on sequence-dependent DNA-templated formation of fluorescent CuNPs [9,14,17]. Interestingly, our proposed method was the utilization of the exceptional target-mediated conformational structure by forming a stable 1:1 complex between actin and DNase I [18]. In this complex, DNase I activity is nearly lost (by about 95% at equimolar ratios), and actin can hardly repolymerize even in the presence of high salt (the binding constant, $K_a = 10^9\ M^{-1}$) [19]. This highly specific interaction has been employed to detect and even purify actin by affinity chromatography [20,21]. Inspired by these, the fluorescent intensity from the *in situ* formation of DNA-CuNPs in digestion reaction may reflect increases and decreases in actin concentration. To the best of our knowledge, this method is the first example of employing environmental-friendly nanoparticles as fluorescent indicators to qualitative and quantitative detection of actin.

2. Experimental

2.1. Materials and instruments

All chemicals used in this work were of analytical grade and directly used without additional purification. Tris (hydroxyamino) methane hydrochloride (Tris-HCl), sodium chloride, magnesium sulfate, calcium chloride, copper sulfate, and ascorbic acid (AA) were purchased from Dingguo Biotech. Company (Beijing, China). Actin, BSA, thrombin, collagen type I, glucose oxidase, IgG, and RPMI 1640 cell medium were purchased from Sigma-Aldrich (Shanghai, China). DNase I were purchased from New England Biolabs Ltd. (USA). Water was deionized and further purified with a Milli-Q water purification system (Millipore, USA), and had an electric resistance > 18.2 MΩ. The HPLC-purified and MS-verified oligonucleotides were purchased from Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). The stock solutions of oligonucleotides purchased were prepared in buffered solutions (10 mM Tris-HCl, 150 mM NaCl, pH 7.6). Substrate DNA were as follows:

5′-ATATATATATATATATATATATATA-3′ /.

3′-TATATATATATATATATATATATAT-5′.

Fluorescence spectra in the range of 500–650 nm were collected on a SHIMADZU RF-5301PC spectrometer under an excitation wavelength of 340 nm. High-resolution transmission electron microscopy (HR-TEM) images were collected on a JEOL 2100 F microscope operating at 200 kV. Time-resolved fluorescence decay curves were collected by using an Edinburgh Analytical Instrument OB920 single-photon counting fluorometer with a pulsed microsecond nitrogen lamp as excitation source.

2.2. Actin assay

Actin assay was carried out in the 500 μL of Tris-HCl buffered solutions (10 mM Tris-HCl, 2.5 mM $MgCl_2$, 0.5 mM $CaCl_2$, 500 nM dsDNA, pH 7.6) and RPMI 1640 cell medium. First, various concentrations of actin were pre-incubated with 0.2 U mL^{-1} DNase I at 37 °C for 3 min. Subsequently, the cleavage reaction was stayed at 37 °C for 30 min, and was ended by quickly heating at 95 °C for 5 min, followed by cooling down to the room temperature. All of experimental processes were achieved by the temperature regulating device of PCR machine. Finally, fluorescence signal of the sensing system was recorded by the formation of DNA-CuNPs after addition of copper ions and AA. Details about the preparation of DNA-CuNPs as well as other experimental information were located in the Supplementary materials section.

3. Results and discussion

3.1. Feasibility verification of the fluorescent strategy

The proposed fluorescent method is shown in Scheme 1. It has been accepted that the size of DNA-CuNPs along with its fluorescence intensity can be regulated by the length of DNA [13]. Substrate DNA can serve as an efficient template for the formation of CuNPs with an excellent fluorescence, whereas DNA fragments fail to support the formation of fluorescent CuNPs, which has been developed for determining nucleases activity, such as S1 nuclease, Exo III and DNase I [11,14,22,23]. In our design, substrate DNA can hardly be cleaved into fragments in the presence of actin, because the stable complex between actin and DNase I can prohibit the digestion. Therefore, a strong emission by the *in situ* formation of CuNPs can be observed. On the contrary, in the absence of actin, fluorescent signal will significantly reduce because of the existence of shorter dsDNA or mono-nucleotides. Accordingly, the sensing systems for actin detection will be established by recording the variation of fluorescence intensity.

Firstly, following the design, it was crucial to obtain efficient and appropriate templates for preparing DNA-CuNPs. Therefore, we carefully investigated the sequence and length of substrate DNA under enzymatic buffered conditions (10 mM Tris-HCl, 2.5 mM $MgCl_2$, 0.5 mM $CaCl_2$, 500 nM dsDNA, pH 7.6). Fluorescence intensity could be used to evaluate the effects of different templates for the formation of fluorescent DNA-CuNPs upon excitation with 340 nm UV lights [13]. As shown in Fig. S1, fluorescent intensity was still quite sensitive to the length of DNA. Besides, AT-rich sequence could induce higher fluorescence than the same length of random sequence. Thus, in order to obtain a relatively high sensitivity and broad dynamic range, the 25 bases of AT-rich sequence (AT25) was eventually adopted as substrate