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Direct monitoring of enzymatic cleavage and facile chemiluminescence strategy for sensitive detection of nuclease activity

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ABSTRACT

The enzymatic cleavage of DNA by nucleases is the origin of many biological processes including genetic engineering, and monitoring sensitively the enzymatic cleavage of DNA is of great significance for life science. Herein, a new CL strategy has been developed for the sensitive, convenient and direct monitoring of the cleavage of ssDNA by specific S1 nuclease based on quite different CL response of (+)AuNPs to long ssDNA and fragmented DNA generated from the enzymatic cleavage reaction of DNA. Strong signal amplification of (+)AuNPs and the thorough and effective hydrolysis reaction of ssDNA render this method to exert remarkable assay performance for detection of S1 nuclease activity with high sensitivity (the detection limit was as low as 6.5×10^{-9} U/μL) and wide linear response range from 0.02×10^{-6} U/μL to 2×10^{-6} U/μL. Together with homogeneous analysis format of "incubate-and-monitor" in the strategy and the simple and easy preparation of signal indicator used for the assay, the present CL assay for probing the enzymatic cleavage process of ssDNA and detecting S1 nuclease activity has displayed convenient operability with low cost. Such excellent feature implied the potential application for monitoring DNA cleavage and facile and sensitive detection of nuclease activity. What is more, based on the inhibition of enzymatic cleavage of DNA, it also provides a promising candidate technique for drug screening and discovery in clinical diagnostics.

1. Introduction

Nucleases are one kind of the most important enzymes that can cleave and hydrolyze the internal phosphodiester linkages of nucleic acid backbone. Many important molecular biology and biotechnology, such as molecular cloning, DNA replication, recombination, and repair can be achieved relying on enzymatic cleavage process of DNA by nucleases [1–4]. In addition, nucleases have also been widely used as a technical tool in promising pharmacological development and gene analysis [5–7]. Therefore, the assay of nuclease activity is of great significance to life science. The commonly used approaches for detection of nuclease activity are gel electrophoresis [8], high performance liquid chromatography (HPLC) [9,10], sedimentation [11], and enzyme-linked immunosorbent assay (ELISA) [12]. These approaches are suffering from high labor intensity, time-consuming, and safety problems of radio labeling. The developed fluorescence methods generally need label using fluorophore or conjugated polymers, which are complicated, expensive and difficult to synthesize labelled substance [13–18]. Recently, colorimetric analysis based on nanomaterials' optical effect has also been developed to use the detection of nucleases [19–21], and the convenience of visual inspection is the prominent characteristics of colorimetry. Although promising, poor sensitivity is serious obstacle to restrict its development. So far, it still remains a challenge to develop facile, cheap, sensitive and convenient strategies for nuclease assay and probe the enzymatic cleavage process of DNA by nucleases.

In chemiluminescence (CL), light emission is from a chemical reaction. CL has become an important analysis technology because of its remarkable advantages like low background, high sensitivity, simple instrumentation, and cheap reagent [22–24]. In recent years, CL nanometer analysis platform builded by means of nanometer materials leads CL analysis into a new stage [25,26]. The signal amplification from nanometer system can effectively improve the sensitivity of assay and realize the high sensitive detection of ultra-low concentration targets including DNA, protein and amino acid [27–32]. Among various signal amplification strategies for achieving higher sensitivity, the charge effect with the help of nanometer material itself performance is found to be a promising signal amplification model [30,33,34]. We and others have utilized this signal amplification of charge effect to construct successfully label-free and sensitive CL aptasensor and fantastic CL sensing system for detecting DNA [30,33,34]. Unfortunately, the research about CL detecting nuclease is few, and CL assay for nuclease

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activity based on signal amplification of nanomaterials' own effect is scarcely reported.

Recently, some colorimetric assays based nanomaterials were developed for the detection of nuclease activity utilizing that during enzymatic cleavage reaction of DNA, single-stranded DNA (ssDNA) and fragmented DNA after enzymatic cleavage can induce nanomaterials to show different optical effect [19–21,35]. In 2006, Wang et al. designed polythiophene derivative-based colorimetric assay nucleases through the conformational change of polythiophene [35]. In 2011, naked-eye sensitive detection of nuclease activity was developed by using gold nanoparticles as probes [21]. Enlightened by this principle of colorimetric assay, herein, we use the charge effect for luminol and H_2O_2 CL reaction exhibited by different action behaviors between positively-charged gold nanoparticles ((+)AuNPs) and ssDNA or fragmented DNA, to monitor the cleavage process of DNA by nucleases, at the same time to realize high sensitive, convenient and practical detection of nuclease activity. Taken (+)AuNPs as the probe of CL response and S1 nuclease as a model enzyme, the "proof-of-idea" of our design can be verified. This mighty signal amplification by nanoparticles' charge effect is presented in the research. In our design, ssDNA digestion process by S1 nuclease and CL reaction for detection of S1 nuclease activity are independent of each other so that the two reaction process can conduct in their respective optimized conditions. As a consequence, both complete and effective cleavage process of DNA by nucleases and ideal signal amplification of (+)AuNPs charge effect result in the proposed CL detecting S1 nuclease activity with super-high sensitivity. The detection limit of S1 nuclease activity with this method could be as low as 6.5×10^{-9} U/μL. Signal amplification by (+)AuNPs' charge effect in this assay is direct, which makes the signal amplification more effective and the method simpler. Response probe used in present assay, (+)AuNPs, is easy to prepare and has low cost. For CL technology itself, the testing cost is also very low. All of these features make the proposed method more practical, especially suitable for application in developing countries. Hence, the developed method has the outstanding sensitivity, convenience and practicability, which enable to address the current facing challenge of detection of nuclease activity.

2. Experimental section

2.1. Chemicals and apparatus

Chloroauric acid (HAuCl4), cysteamine and sodium borohydride for synthesis of (+)AuNPs were purchased from Sigma (St. Louis, MO, USA). The 25-mer ssDNA (5′-GAG TTA GCA CCC GCA TAG TCA AGA T-3′) is used as the nuclease substrate, which was HPLC-purified and synthesized by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). The DNA solution was prepared in TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.5) and stored at 4 °C. S1 nuclease, phosphodiesterase (E2), DNase, RNase, exonuclease III (Exo III), BSA, thrombin and AchE were obtained from Sigma (St. Louis, MO, USA). ATP, inhibitor of S1 nuclease, was purchased from Sigma (St. Louis, MO, USA). Luminol was also obtained from Sigma (St. Louis, MO, USA). Luminol was dissolved in NaOH (0.10 M) solution to prepare stock solution (25 mM) and then diluted with water, which was stored in the dark for 1 week prior to use. Hydrogen peroxide (H_2O_2, 30%) was obtained from Fuzhou Chemical Reagent Plant (Fuzhou, China). The working H_2O_2 solutions were prepared fresh daily from 30% (w/w) H_2O_2. All other solvents and reagents in this research were of analytical grade and used without further purification. Millipore water (18 MΩ cm) was used in all experiments.

CL was measured and recorded with a model IFFM-D chemiluminescence analyzer (Xi'an Remex analysis instrument Co. Ltd., Xi'an, China). Zeta potentials were recorded with a Malvern Zetasizer 2000 (Malvern, England). The transmission electron microscopy (TEM) images of AuNPs were taken by using a JEM-2100 TEM (Japan Electronics Co., Ltd). UV–visible adsorption spectra were recorded on a Hitachi U-3900H UV/Vis Spectrophotometer (Tokyo, Japan) at room temperature using a 500 μL black-body quartz curette with 1 cm path length. An HH-1 electric-heated thermostatic water bath (Beijing Kewen Instrumental Factory, Beijing, China) was used to control the reaction temperature at 0.1 °C intervals. The pH measurements were carried out on model PB-10 digital ion analyzer (Sartorius Scientific instruments Co., Ltd., China, Beijing).

2.2. Synthesis and characterization of positively charged AuNPs

(+)AuNPs, the signal response element for this CL strategy, were prepared according to the published procedure [36]. Before synthesis, all glassware and magnetic stirrer bars used in the following procedure were soaked in freshly prepared aqua regia (HCl/HNO_3 = 3:1, v/v) for 24 h, thoroughly rinsed in ultrapure water, and dried in air prior to use, to avoid unwanted nucleation during the synthesis in order to ensure the fine preparation of (+)AuNPs. Briefly, 400 μL cysteamine solution (0.2 M) was added to 40 mL of 0.014 M $HAuCl_4$ solution and stirred completely for about 20 min at room temperature. 10 μL of 0.01 M sodium borohydride solution was added, and the mixture was vigorously stirred for 15 min at room temperature in the dark. Once the solution changed to wine-red, the resulting wine-red solution was further stirred for another 20 min. Then, the as-prepared (+)AuNPs was stored in the refrigerator (4 °C) until needed. The as-prepared (+)AuNPs were charactered with TEM analysis and UV–Visible absorption spectra. The analysis results of TEM showed that their average size was about 34 nm.

2.3. Procedure for DNA cleavage by S1 nuclease

A typical DNA cleavage process by S1 nuclease was realized by the following procedure. 50 μL of ssDNA solution (6×10^{-7} M) and 50 μL of different concentration S1 nuclease were mixed. The mixed solution was incubated in digestion buffer solution (2 mM CH_3COONa, 15 mM NaCl, 0.1 mM $ZnSO_4$, pH 4.6) for 30 min at 37 °C to complete DNA hydrolyze.

2.4. CL probing of enzymatic cleavage and CL detection of S1 nuclease

The whole procedure for CL probing enzymatic cleavage and CL detecting S1 nuclease could be divided into two steps: the action process of (+)AuNPs and the triggering of CL signal. First, to the above DNA digestion product mixture solution, 200 μL of (+)AuNPs solution was added to interact with ssDNA in Britton Robinson (BR) buffer solution (pH = 3.6) for 5 min at room temperature (ca. 20 °C). Second, CL probing and measurements were performed at room temperature by means of static injection. After action of (+)AuNPs, 50 μL of the above resulting digestion product solution was decanted to the quartz cuvette (used as CL reactor), and the CL reaction was triggered by immediately injecting 200 μL of luminol–H_2O_2 CL reagents (the volume ratio of 6.0×10^{-4} M luminol and 1.0×10^{-3} M H_2O_2 was 2:1). The CL signal was measured and recorded with the IFFL-D chemiluminescence analyzer. Thus, responded CL signal can probe enzymatic cleavage of ssDNA by S1 nuclease and S1 nuclease concentration was quantified by the CL peak intensity.

3. Results and discussion

3.1. Design principle of direct CL probing for DNA cleavage

This design of CL monitoring DNA cleavage process by nuclease and simultaneously detecting nuclease activity is according to (+)AuNPs' different CL response on ssDNA and fragmented DNA from enzymatic cleavage in the luminol–H_2O_2 CL reaction. Via the charge effect of (+)AuNPs for luminol CL reaction, the assay design will effectively amplify the identification of (+)AuNPs for long DNA and fragmented