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Label-free fluorometric detection of S1 nuclease activity by using polycytosine oligonucleotide-templated silver nanoclusters

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ABSTRACT

S1 nuclease has an important function in DNA transcription, replication, recombination, and repair. A label-free fluorescent method for the detection of S1 nuclease activity has been developed using polycytosine oligonucleotide-templated silver nanoclusters (dC_{12}–Ag NCs). In this assay, dC_{12} can function as both the template for the stabilization of Ag NCs and the substrate of the S1 nuclease. Fluorescent Ag NCs could be effectively formed using dC_{12} as the template without S1 nuclease. In the presence of S1 nuclease, dC_{12} is degraded to mono- or oligonucleotide fragments, thereby resulting in a reduction in fluorescence. S1 nuclease with an activity as low as 5×10^{-8} U μl^{-1} (signal/noise = 3) can be determined with a linear range of 5×10^{-7} to 1×10^{-3} U μl^{-1}. The promising application of the proposed method in S1 nuclease inhibitor screening has been demonstrated using pyrophosphate as the model inhibitor. Furthermore, the S1 nuclease concentrations in RPMI 1640 cell medium were validated. The developed method for S1 nuclease is sensitive and facile because its operation does not require any complicated DNA labeling or laborious fluorescent dye synthesis.

S1 nuclease hydrolyzes single-stranded DNA (ssDNA)[1] or RNA into 5′-phosphomononucleotide and 5′-phosphooligonucleotide. This enzyme has been widely used in various applications such as in the determination of nucleic acid structure, the quantitation of nucleic acid hybridization, mapping mutations, detection of gaps in duplex DNA, and studying the interactions of DNA with various intercalating agents [1–3]. S1 nuclease has been particularly used to probe the disruption of the DNA structure by numerous carcinogens and antimitotic drugs [4]. Therefore, detecting S1 nuclease activity and screening for its potential inhibitors is substantially important.

Numerous traditional methods have been used to measure the nuclease activity [5]. Although these methods are generally accurate, their routine use in laboratory is restricted because of their laborious and complicated conjugated chemistries. Recently, a number of colorimetric [6], electrochemical [7,8], and fluorescent [3,9,10] assays for S1 nuclease detection have been developed. In addition, advanced materials, such as gold nanoparticles [6], carbon nanotubes [11], perylene derivatives [3], distyrylanthracene [10], and cationic polythiophene derivative [12], have been used for the construction of novel assay strategies for S1 nuclease. However, these strategies have specific drawbacks such as low detection sensitivity, expensive materials, and time-consuming assay procedures. Therefore, developing a highly sensitive and facile method for detecting S1 nuclease activity and screening for its potential inhibitors is substantially important.

Noble metal nanoclusters (NCs) have been used in various applications, such as bioimaging, biosensing, and specific protein detection, because of their ultrafine size, nontoxicity, and good biocompatibility [13–21]. NCs can be routinely prepared in aqueous solutions with a number of thiolated or polymeric templates, including thiolated compounds [22], DNAs [23,24], proteins [25], and dendrimers [26,27]. DNA is a particularly interesting ligand for preparing fluorescent metal NCs and developing biosensors [28]. For the detection of S1 nuclease activity, two interesting DNA-templated NC methods, namely the fluorometric assay based on double-strand [29] or single-strand [30] DNA-templated copper (Cu) NCs/NPs (nanoparticles), have been recently developed. However, the relatively low quantum yield of Cu NCs/NPs (e.g., a value of 0.068 for T30-templated Cu NCs/NPs) [30] indicates that the detection sensitivity of these methods could be further improved.

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[1] *Abbreviations used:* ssDNA, single-stranded DNA; NC, nanocluster; Cu, copper; NP, nanoparticle; dC_{12}, polycytosine oligonucleotide; Ag, silver; UV–Vis, ultraviolet–visible; PB, phosphate buffer.

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In this study, polycytosine oligonucleotide (dC_{12}) was used as both the S1 nuclease substrate and template for the formation of highly fluorescent dC_{12}–Ag (silver) NCs. S1 nuclease degrades dC_{12} to nucleotide fragments, thereby resulting in a reduction in fluorescence. Thus, a label-free and highly sensitive method for S1 nuclease activity assay was developed.

Materials and methods

Materials and apparatus

The S1 nuclease was provided by TaKaRa Biotechnology (Dalian, China). Oligonucleotides with dC_{12} were synthesized and purified by Sangon Biotechnology (Shanghai, China). The oligonucleotide stock solutions (200 μM) were prepared with deionized water and kept frozen at −20 °C. The RPMI 1640 cell medium was also purchased from Sangon Biotechnology. All other chemicals were of analytical grade and used without further treatment. Deionized water was prepared using a Millipore Milli-Q water purification system (18.2 MΩ). The ultraviolet–visible (UV–Vis) and fluorescence spectra of DNA–Ag NCs were obtained using a PerkinElmer Lambda 35 spectrometer and a PerkinElmer LS 55 spectrometer, respectively. Mass spectra were acquired in negative ion mode with 2.5-kV needle and 40-V cone voltages.

Determination of S1 nuclease and inhibitory detection

In total, 100 μl of the mixtures containing 20 μM dC_{12} and various concentrations of S1 nuclease ranging from 0.0 U μl^{-1} to 5×10^{-2} U μl^{-1} was incubated in an enzyme reaction buffer (30 mM CH_3COONa, 100 mM $NaNO_3$, and 1 mM $Zn(NO_3)_2$, pH 4.6) at 37 °C for 0.5 h in the PCR (polymerase chain reaction) tube. After enzyme digestion, the mixture was heated at 95 °C for 10 min to terminate the cleavage reaction. The solutions of sodium phosphate buffer (PB; 5 mM, pH 7.0, 92 μl) and $AgNO_3$ (2 mM, 6 μl) were then separately introduced and further incubated in an ice bath for 15 min. Lastly, freshly prepared $NaBH_4$ (5 mM, 2.5 μl) by dissolving $NaBH_4$ powder in deionized water was added to induce fluorescent DNA–Ag NC formation. After being kept in the dark for 2 h at room temperature, the mixtures were diluted with PB for subsequent fluorescence measurements. The 10-fold amount (1×10^{-2} U/μl) of *Eco*RI, acid and alkaline phosphatase, *Exo*III, and *Escherichia coli* ligase in the mixture was used to evaluate the specificity of the fluorometric assay to the S1 nuclease. The inhibition experiments were the same as for the above procedure except for the 15-min preferential incubation of the inhibitor with the S1 nuclease before the addition of dC_{12}.

Dynamic detection of S1 nuclease

A series of 100-μl reaction mixtures containing a fixed concentration of 20 μM dC_{12} and 5×10^{-3} U μl^{-1} S1 nuclease were incubated at 37 °C for 0, 1, 2, 3, 4, 5, 7, 10, 15, 20, and 30 min. After incubation for a predetermined period, the following experiments were performed according to the aforementioned procedure.

Detection of S1 nuclease in complex samples

To test the practicality of the proposed strategy, the target from the complex samples was analyzed according to the methods presented in the literature [30]. Different amounts of S1 nuclease were spiked into RPMI 1640 cell medium, in which 1 mM zinc nitrate was added to protect the enzyme activity. The S1 nuclease was subsequently detected via the proposed method, and the recovery value was determined.

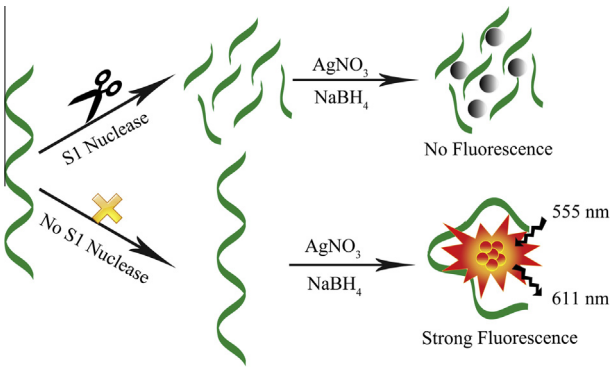
Results and discussion

Detection mechanism of the method

Scheme 1 illustrates the principle of the Ag NC-based fluorescent sensing system for S1 nuclease. The assay mechanism was based on function of dC_{12} as both the S1 nuclease substrate and template for the stabilization of Ag NCs. The oligonucleotide dC_{12} was selected as substrate because the dC_{12}-templated silver clusters exhibit excellent fluorescence properties and have been well characterized by Ritchie and coworkers [31] and successfully used in bioanalysis [32]. In the absence of S1 nuclease, the strong fluorescent dC_{12}–Ag NCs could be formed through the reduction of Ag^+ by $NaBH_4$. However, dC_{12} could be digested into mono- and/or small fragments by the S1 nuclease, which results in the failed formation of Ag NCs because of the lack of a suitable template. Thus, S1 nuclease activity can be successfully determined through the change in the fluorescence of dC_{12}–Ag NCs.

Characteristics of Ag NCs and feasibility of the method

The dC_{12}–Ag NCs were synthesized according to the procedures in the literature [31,32]. To confirm the formation of the fluorescent dC_{12}–Ag NCs, both UV–Vis and fluorescence spectra were analyzed. The absorbance spectra (Fig. 1, curve a) show two dominant peaks at maximum absorbance wavelengths of 443



Scheme 1. Schematic diagram of S1 nuclease activity assay based on dC_{12}–Ag NCs.

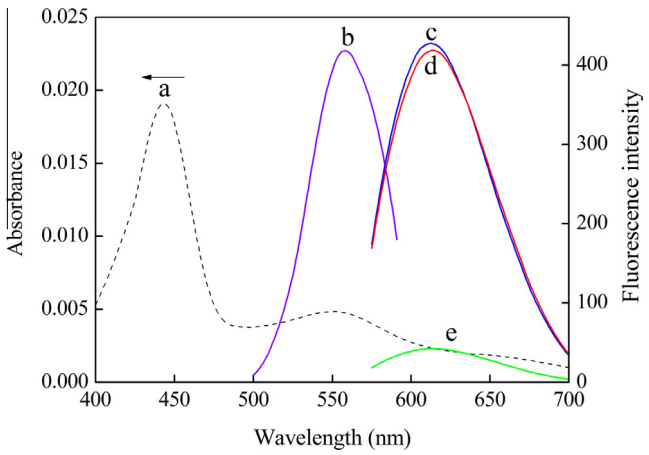


Fig.1. UV–Vis (a) and fluorescence spectra of dC_{12}–Ag NC probe: excitation curve (b), emission curve (c), incubation with inactivated S1 nuclease (d), and incubation with activated S1 nuclease (e). Maximum wavelengths of excitation and emission are 555 and 611 nm, respectively.