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A label-free DNA-templated silver nanocluster probe for fluorescence on–off detection of endonuclease activity and inhibition

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ABSTRACT

Endonuclease cleavage of DNA plays an important role in biological and medicinal chemistry. This study aimed to develop a reliable and sensitive method for nuclease activity assay by combining the high specificity of DNA cleavage reactions with ultrahigh fluorescence turn-on abilities of guanine-rich (G-rich) DNA sequences in proximity to silver nanoclusters (Ag NCs). The DNA-templated Ag NC (DNA-Ag NC) probe with endonuclease recognition sequence consists of NC and a G-rich probe. The NC probe was designed by adding Ag NC nucleation sequence at the 5′-end. The G-rich probe is the complementary DNA sequence modified by adding a G-rich overhang sequence at the 3′-end. Thus, the fluorescence of DNA-Ag NC probe was activated because of DNA hybridization. When these DNA-Ag NC probes were exposed to the targeted endonucleases, specific DNA cleavages occurred, and pieces of G-rich DNA fragments separated from Ag NCs, resulting in fluorescence turn-off. The endonuclease activity was quantified by monitoring the change in the fluorescence intensity. Detection was demonstrated by assaying EcoRI activity. Under optimized conditions, the fluorescence reduction efficiency was linear with the EcoRI concentration in the range of 5.0×10^{-4} U μL^{-1} to 3.0×10^{-3} U μL^{-1}, with a detection limit of 3.5×10^{-4} U μL^{-1}, which is much better than or at least comparable with that in previous reports. The potential application of the proposed method for screening endonuclease inhibitors was also demonstrated. The presented assay protocol proved to be convenient, effective, sensitive, and easy in preparing the fluorescent probe.

1. Introduction

Endonucleases belong to the family of nucleases that can hydrolyze the phosphodiester linkages in the nucleic acid backbone. They play a vital role in many important biological processes involving DNA replication, recombination, repair, genotyping, mapping, molecular cloning, and polymerase chain reaction assay (Gite and Shankar, 1995; Lieber, 1997; Grindley et al., 2006; Ma et al., 2000; Pingoud and Jeltsch, 2001). Moreover, they are recognized as promising targets for antimicrobial and antiviral drug development (Heithoff et al., 1999; Xu et al., 2007; Robertson and Wolffe, 2000; Shames et al., 2007; Brueckner and Lyko, 2004). EcoRI, which belongs to type II restriction endonuclease, is considered part of a defense system that protects living cells against foreign DNA by recognizing and cleaving a defined DNA sequence GAATTC. A facile analysis of activity and inhibition is pivotal in the fields of modern molecular biology (Huang et al., 2011a; Liu et al., 2011). Traditional methods, such as gel electrophoresis, chromatography, colorimetry, ^{32}P-radioactive labeling, and ELISA, among others, have been established for assaying nuclease activities (VanderVeen et al., 2005; Alves et al., 1989; Song et al., 2009; Li et al., 2010; Wright et al., 1999; Jeltsch et al., 1993). However, these protocols are time-intensive, DNA-consuming, laborious, insensitive, and discontinuous, and they require radioactive labeling of substrates (Huang et al., 2011b). Fluorescence methods (Zhou et al., 2012) were developed for assaying endonucleases, for example, fluorescence detection using molecular break light and beacon DNA probes (Li et al., 2000; Ma et al., 2007; Kettling et al., 1998; Biggins et al., 2000; Li et al., 2007; Liu et al., 2006) and fluorescence resonance energy transfer-based assay (Feng et al., 2009; Deng et al., 2012). Nanoparticle-enhanced fluorescence polarization assay for measuring endonuclease activity has been demonstrated recently (Huang et al., 2011a). However, many organic fluorophores suffer the drawbacks of low photostability and quantum yields, which relatively narrow the absorption along with broad emission spectra and small Stokes shifts (Huang et al., 2011b).

Recently, the interest in fluorescent silver nanocluster (Ag NC) synthesis and its application in the area of bioassays has increased (Chen et al., 2011; Liu et al., 2012), especially in fluorescent Ag NCs using DNA as scaffolds in aqueous solution (Zhang and Ye, 2011; Zhang et al., 2012; Richards et al., 2008; Han et al., 2012; Park et al., 2013; Maclean et al., 2013). The fluorescence emission bands of Ag NCs

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stabilized by oligonucleotide can be tuned throughout the visible and near infrared range with different oligonucleotide sequences (Richards et al., 2008; Petty et al., 2004). Moreover, the ultrasmall size of NCs leads to discrete and size-dependent electronic transitions and interesting molecular-like properties, such as strong fluorescence and quantized charging (Latorre and Somoza, 2012). Werner and colleagues reported an interesting phenomenon that dark DNA-templated Ag NCs (DNA-Ag NCs) could be transformed into bright red-emitting clusters enhanced 500-fold when NCs are in proximity to guanine-rich (G-rich) DNA sequences (Yeh et al., 2010). A series of experiments were performed to explain the mechanism of fluorescence enhancement. However, the physical mechanism driving the increase in red fluorescence emission cannot be fully understand yet (Yeh et al., 2010). Evidently, they successfully developed a turn-on NC beacon for DNA detection and identification of a number of disease-related single-nucleotide polymorphisms (Yeh et al., 2012). Such a modification-free and separation-free format greatly simplifies DNA detection. Furthermore, the sensitive detection of protein (Li et al., 2012), adenosine triphosphate, and adenosine deaminase (Zhang et al., 2012) was established using DNA-Ag NC probes that fluoresce upon the effects of G-rich DNA proximity.

Based on the above facts, we designed and synthesized a novel, label-free, and highly sensitive fluorescent DNA-Ag NC probe for EcoRI activity and inhibition using a cytosine-rich single-stranded DNA with the sequence of EcoRI recognition site, as illustrated in Scheme 1. The fluorescence of the DNA-Ag NCs can be strongly enhanced when Ag NCs are in proximity to G-rich DNA sequences of another designed DNA strand. Therefore, the DNA-Ag NC probe can be used to detect the specificity of the DNA cleavage reaction using the fluorescence on–off of Ag NCs.

2. Experimental

2.1. Reagents and materials

Endonucleases were purchased from Sangon Biotech Co. (Shanghai, China). The oligonucleotides designed in this study were all synthesized and HPLC purified by Sangon Biotech Co. (Shanghai, China) and were used as received.

Other chemicals were of analytical grade and used without further purification. The oligonucleotide stock solutions were prepared with 5 mM phosphate buffer (pH 7.0) and kept frozen at −20 °C. Millipore MilliQ (18 MΩ cm) water was used in all experiments. About 50 mM Tris–HNO_3 solution (pH 7.5) containing 50 mM $NaNO_3$ and 10 mM $Mg(NO_3)_2$ was prepared for EcoRI cleavage buffer.

2.2. Apparatus

Ultraviolet–visible (UV–vis) absorption spectra were measured using UV–vis 2450 made by Shimadzu Co., Ltd. Fluorescence measurements were performed using the Cary Eclipse Fluorescence Spectrophotometer. The emission spectra of each system were measured at the excitation wavelength of 575 nm and emission range of 585–700 nm.

2.3. DNA-Ag NC preparation

The synthesis of DNA-Ag NCs was based on the reported method with minor modifications (Lan et al., 2011; Ritchie et al., 2007). Briefly, DNA solutions were prepared by adding the DNA stock solutions to phosphate buffer (5 mM, pH 7.0). To prepare DNA-Ag NCs, aliquots of $AgNO_3$ solution (2 mM, 30 μL) were added separately to one of the DNA solutions (0.2 mM, 50 μL) to provide an Ag^+-to-DNA molar ratio of 6:1. This molar ratio enabled the preparation of DNA-Ag NCs at their most fluorescence. After incubation in an ice bath for 15 min, the mixtures were reduced by vigorously shaking with freshly prepared $NaBH_4$ solution (5 mM, 12 μL) for approximately 30 s. The mixtures were kept for 2 h at room temperature and then stored in the dark overnight at 4 °C. The final concentrations of DNA, $AgNO_3$, and $NaBH_4$ were 10, 60, and 60 μM, respectively. The DNA-Ag NCs were diluted with 50 mM Tris–HNO_3 solution (pH 7.5) containing 50 mM $NaNO_3$ and 10 mM $Mg(NO_3)_2$ buffer to reach the appropriate concentration for further use.

2.4. Assay of EcoRI endonuclease activity and inhibition

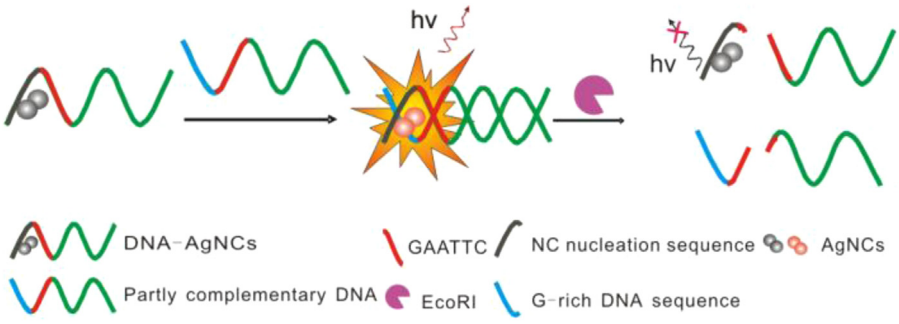
Appropriate blanks corresponding to the buffer were subtracted to correct the fluorescence background. All experiments were triplicated and found to be reproducible within experimental errors. First, the mixture of equimolar DNA-Ag NCs and the partly complementary DNA was heated to 95 °C for 1 min and gradually cooled to 37 °C (Yeh et al., 2010). Second, the mixture was diluted 20-fold with buffer solution (50 mM Tris–HNO_3, 50 mM $NaNO_3$, 10 mM $Mg(NO_3)_2$, pH 7.5), and the fluorescence was measured after the mixture was incubated at 37 °C for 60 min. Finally, EcoRI endonuclease was added to the mixture to study EcoRI–DNA interactions by monitoring the change in fluorescence intensity.

The inhibitor 5-fluorouracil of EcoRI was incubated with 0.5 μM DNA1-based Ag NC probe in Tris–HNO_3 buffer for 15 min at 37 °C. Then, EcoRI was added, and the resulting solution was incubated at 37 °C for 20 min. The detection procedure was the same as shown in the aforementioned assay of EcoRI endonuclease activity.

3. Results and discussion

3.1. Principle of Ag NCs-based nanoprobes

In this study, we used DNA-Ag NCs to detect the EcoRI endonuclease activity and inhibition. The assay mechanism is shown in Scheme 1. In this assay, two single-stranded DNAs were rationally designed. One was modified by adding a sequence of EcoRI recognition site GAATTC and a Ag NC nucleation sequence at the 5′-end. The other



Scheme 1. Schematic diagram showing the principle of fluorescence on–off assay for EcoRI activity.