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A label-free fluorescent assay for deoxyribonuclease I activity based on DNA-templated silver nanocluster/graphene oxide nanocomposite

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ABSTRACT

A novel label-free system for the sensitive fluorescent detection of deoxyribonuclease I (DNase I) activity has been developed by utilizing DNA-templated silver nanocluster/graphene oxide (DNA-AgNC/GO) nanocomposite. AgNC is first synthesized around C-rich template DNA and the resulting DNA-AgNC binds to GO through the interaction between the extension DNA and GO. The resulting DNA-AgNC/GO would show quite reduced fluorescence signal because the fluorescence from DNA-AgNCs is quenched by GO. In the presence of DNase I, however, it degrades the DNA strand within DNA/RNA hybrid duplex probe employed in this study, consequently releasing RNA which is complementary to the extension DNA. The released free RNA then extracts DNA-AgNC from GO by hybridizing with the extension DNA bound to GO. This process would restore the quenched fluorescence, emitting highly enhanced fluorescence signal. By employing this assay principle, DNase I activity was reliably identified with a detection limit of 0.10 U/ml which is lower than those from previous fluorescence-based methods. Finally, the practical capability of this assay system was successfully demonstrated by its use to determine DNase I activity in bovine urine.

1. Introduction

Deoxyribonuclease I (DNase I) is a non-restriction endonuclease which hydrolyzes phosphodiester bonds of single- or double-stranded DNA to yield short oligonucleotides. DNase I activity degrades DNA released into blood after death of cells, thereby maintaining the physiological level of DNA in the blood (Cherepanova et al., 2007; Lee et al., 2001). The lack of DNase I activity has been found in the patients suffering from systemic lupus erythematosus (SLE), xeroderma pigmentosum and cancers including lymphoma malignum, stomach cancer, and colon cancer (Economidou-Karaoglou et al., 1988; Napirei et al., 2000; Tamkovich et al., 2006; Wu et al., 2013). On the other hand, the elevation of DNase I activity is closely associated with diabetes and cancers such as oral cavity cancer and breast cancer (Economidou-Karaoglou et al., 1988; Spandidos et al., 1980; Zhu et al., 2014), and has been used as a diagnostic indication for the early prognosis of acute myocardial infarction and myocardial ischemia (Arakawa et al., 2005; Kawai et al., 2004; Morikawa et al., 2007).

Due to the biological and clinical significance of DNase I, there have been various methods developed for the determination of DNase I activity, which include single radial enzyme diffusion (SRED) method (Nadano et al., 1993), enzyme-linked immunosorbent assay (ELISA) (Nakajima et al., 2009), electrochemical assay (Sato et al., 2009), microchip electrophoresis (Fujihara et al., 2011), colorimetric method (Xu et al., 2007), and fluorescent method (Choi and Szoka, 2000; Dou and Yang 2013; Sun et al., 2013; Zhou et al., 2012). Of these, fluorescence-based methods have been extensively developed due to their several advantages including high sensitivity, simplicity, and ease of operation. However, these strategies still have the drawbacks including the costly modification of fluorophores and potential false positive signals. In addition, the sensitivity needs to be further improved. Therefore, it is highly desirable to develop a new fluorescent strategy operated in a both label-free and turn-on manner for the sensitive determination of DNase I activity (Table S1).

In recent years, as a key component to construct a novel fluorescent biosensing strategy, graphene oxide (GO) as a water soluble derivative of graphene has received a growing interest owing to its unique features such as good water dispersibility, facile surface modification, and strong mechanical strength (Dikin et al., 2007; Liu et al., 2008; Mohanty and Berry, 2008). GO binds to DNA through π-π stacking and hydrophobic interactions of its hexagonal rings with DNA nucleobases, and has an excellent quenching ability with the long-range nanoscale energy transfer

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(He et al., 2010; Varghese et al., 2009). These properties have tremendously contributed to the development of biosensing platforms with remarkable sensitivity (Cui et al., 2015; Huang et al., 2014; Jang et al., 2013; Ling et al., 2016; Zhang et al., 2016a). Most of the sensing platforms have been designed based on the fact that the single-stranded (ss) DNA exhibits the stronger affinity to GO than its duplex (Cui et al., 2015; Huang et al., 2014; Jang et al., 2013; Ling et al., 2016; Zhang et al., 2016a). These systems, however, inevitably require the complicated and expensive labeling process of organic fluorophores. As a compelling alternative to organic fluorophore, DNA-templated silver nanoclusters (DNA-AgNCs) which possess outstanding spectroscopic and photophysical properties, low toxicity, and biocompatibility (Cao et al., 2015; Park et al., 2014b; Richards et al., 2008; Vosch et al., 2007; Zhang et al., 2015, 2016b), were recently applied to develop new types of fluorescent biosensors by integrating them into GO (Liu et al., 2013).

By taking advantage of the unique properties of GO and DNA-AgNCs, we herein developed a novel, label-free, and fluorescence turn-on method for the sensitive determination of DNase I activity, which is the first application of DNA-AgNC/GO nanocomposite in the enzyme activity assay. This system employs the RNA/DNA hybrid as a substrate probe for DNase I and DNA-AgNC/GO nanocomposite as a key detection probe in which DNA-AgNC and GO serve as a reporter unit and a superquencher, respectively. The diagnostic applicability of present method has been successfully demonstrated by its application to the determination of DNase I activity present in bovine urine.

2. Materials and methods

2.1. Materials

All nucleic acids used in the present study were synthesized from Bioneer® (Daejeon, South Korea) and purified by polyacrylamide gel electrophoresis, except for RNA strand (purified by Bio-RP) whose stability was ensured for this work (Fig. S1). The sequences of all nucleic acids are listed in Table S2. DNase I, ribonuclease H (RNase H), Hind III, exonuclease I (Exo I), exonuclease III (Exo III), and lambda exonuclease (λ exo) were purchased from New England Biolabs Inc. (Beverly, MA, USA). Graphite, sulfuric acid (H_2SO_4), potassium persulfate ($K_2S_2O_8$), phosphorous pentoxide (P_2O_5), hydrogen peroxide (H_2O_2), potassium permanganate ($KMnO_4$), hydrogen chloride (HCl), silver nitrate ($AgNO_3$), sodium borohydride ($NaBH_4$), sodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), cysteine (Cys), adenosine triphosphate (ATP), and bovine urine were purchased from Sigma-Aldrich. All other chemicals were of analytical grade and used without further purification. Ultrapure DNase/RNase-free distilled water was purchased from Bioneer® (Daejeon, South Korea) and used in all experiments (Park et al., 2014a).

2.2. Preparation of GO

GO was synthesized from natural graphite powder ($< 20\ \mu m$, Aldrich) according to a modified Hummers method (Hummers Jr and Offeman, 1958). The graphite powder (1 g) was put into a solution of concentrated H_2SO_4 (5 ml), $K_2S_2O_8$ (0.5 g), and P_2O_5 (0.5 g) and stirred at 80 °C for 4.5 h. The resulting dark blue mixture was then carefully diluted with 1 L of distilled water, filtered, and washed on the filter to remove all traces of acid. The product was dried in air at ambient temperature overnight. The oxidized graphite was then subjected to further oxidation according to Hummers method. The oxidized graphite powder (1 g) was put into concentrated H_2SO_4 (26 ml), and $KMnO_4$ (3 g) was slowly added with stirring on an ice bath so that the temperature of the mixture was kept below 10 °C. The mixture was then stirred at 35 °C for 2 h, and distilled water (46 ml) was added. In 2 h, the reaction was terminated by the addition of a large amount of distilled water (140 ml) and 30% H_2O_2 solution (2.5 ml), resulting in the color change of the mixture to bright yellow. After precipitation of the mixture for at least one day and removal of the clear supernatant, the precipitated mixture was filtered and washed with 10% (v/v) HCl solution (1 L) in order to remove remaining metal ions. The resulting solid was dried in air and suspended in distilled water, which was subjected to dialysis for 2 weeks to completely remove metal ions and acids. The product was washed several times with distilled water and dried at 50 °C in vacuum oven for one day. Dried GO was then dispersed in water to create a 0.5 mg/ml dispersion and subjected to ultrasonication using a Brandson Digital Sonifier (S450D, 500 W, 39% amplitude) for 40 min to give a stable suspension. The obtained brown dispersion was centrifuged at 4000 rpm for 10 min to obtain stable supernatant GO. The resulting GO was finally characterized by using atomic force microscopy (AFM) and Fourier transform infrared spectroscopy (FT-IR) (Fig. S2).

2.3. Preparation of DNA-AgNCs

DNA-AgNCs were synthesized by following the previously reported procedure with the slight modification (Richards et al., 2008; Yeh et al., 2010). Briefly, 10 μl of DNA template (100 μM) and 20 μl of phosphate buffer (200 mM, pH 7.0) were applied to the 150 μl of distilled water and mixed together. To the mixture, 10 μl of $AgNO_3$ (600 μM) was added and stirred vigorously for 30 s, followed by the incubation at 4 °C for 15 min to form DNA-Ag^+ complex. Then, 10 μl of freshly prepared $NaBH_4$ (600 μM) was added to the solution to induce the reduction. After vigorous shaking for 30 s, the resulting solution was kept in the dark at 4 °C for at least 6 h. Finally, 200 μl of DNA-AgNC (5 μM) in phosphate buffer (20 mM, pH 7.0) was prepared and characterized by TEM analysis (Fig. S3).

2.4. Detection procedure of DNase I activity

The reaction mixtures were separately prepared as part A and part B. Part A (total volume of 25 μl) composed of 2 μM C-RNA, 2 μM S-DNA, and DNase I at varying concentrations in a 1X DNase I reaction buffer (10 mM Tris-HCl, 2.5 mM $MgCl_2$, 0.5 mM $CaCl_2$, pH 7.6) was incubated at 37 °C for 1 h, heated at 80 °C for 15 min to terminate the enzymatic reaction, and cooled slowly to 25 °C (0.1 °C/s), followed by the incubation at 25 °C for 15 min. Part B (total volume of 25 μl) composed of 1 μM DNA-AgNC and 72 μg/ml GO in a 1X DNase I reaction buffer was incubated at 25 °C for 10 min (Fig. S5(a)). The part A and B were then mixed, giving the final solution (total volume of 50 μl) consisting of 1 μM C-RNA, digested S-DNA, 500 nM DNA-AgNC, 36 μg/ml GO, and DNase I in a 1X DNase I reaction buffer. After the incubation of final solution at 25 °C for 1 h, the fluorescence signals were measured.

2.5. Gel electrophoresis analysis of DNase I activity assay products

The reaction products were resolved on 3% agarose gel using 1X TBE as the running buffer at a constant voltage of 100 V for 50 min. After staining with EtBr, gel image was taken with an UV transilluminator.

2.6. Assay for DNase I activity in bovine urine (1%)

Bovine urine was previously centrifuged at 13,000 rpm for 10 min to remove particulate matters and the supernatants were