Biosensors and Bioelectronics 93 (2017) 293–297

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

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

Chang Yeol Lee [a], Ki Soo Park [a], Yun Kyung Jung [b], Hyun Gyu Park [a,*]

[a] Department of Chemical and Biomolecular Engineering (BK 21+ program), KAIST, Daehak-ro 291, Yuseong-gu, Daejeon 305-701, Republic of Korea
[b] Department of Energy and Chemical Engineering, UNIST, UNIST-gil 50, Eonyang-eup, Ulju-gun, Ulsan 689-798, Republic of Korea

ARTICLE INFO

Article history:
Received 17 June 2016
Received in revised form
10 August 2016
Accepted 22 August 2016
Available online 24 August 2016

Keywords:
Deoxyribonuclease I
RNA/DNA hybrid
DNA-templated silver nanocluster
Graphene oxide
Nanocomposite
Fluorescent enzyme assay

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

* Corresponding author.
E-mail address: hgpark@kaist.ac.kr (H.G. Park).

http://dx.doi.org/10.1016/j.bios.2016.08.073
0956-5663/

(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

used to prepare sample solution containing DNase I for further analysis. As-prepared bovine urine was diluted with 1X DNase I reaction buffer, and spiked with DNase I at varying concentrations, which was then subjected to the same procedure to determine DNase I activity as described above.

2.7. Melting curve analysis for C-RNA/S-DNA hybrid

The C-RNA/S-DNA hybrid solution prepared according to the detection procedure of DNase I activity, but excluding DNase I, DNA-AgNC, and GO, was heated at 80 °C for 5 min, cooled slowly to 25 °C (0.1 °C /s), and incubated at 25 °C for 20 min. The resulting fluorescence signal from SYBR green I (Invitrogen, CA, USA) was measured on a C1000™ thermal cycler (Bio-Rad, CA, USA) as the temperature was increased from 25 °C to 80 °C with an increment of 0.5 °C. The first derivative plot [-d(RFU)/dT] was used to determine the melting temperature.

2.8. Instrumentation

The AFM image and height profile of GO were obtained using a XE-100 scanning probe microscope (Park Systems, South Korea) and FT-IR spectrum of GO was obtained using a IFS66V/S & HYPERION 3000 FT-IR microscope (Bruker Optiks, Germany). The prepared DNA-AgNC was analyzed by using a field-emission transmission electron microscopy (Tecnai, FEI, Netherlands) operating at an accelerating voltage of 300 kV. Fluorescence intensities were measured using a Tecan Infinite M200 pro microplate reader (Mnnedorf, Switzerland) and black, 384-well Greiner Bio-One microplates (ref: 781077, Courtaboeuf, France) at the excitation wavelength of 540 nm (Park et al., 2013). The image of gel electrophoresis was obtained by using Gel Doc EZ Imager (Bio-rad, Hercules, USA).

3. Results and discussion

3.1. Overall detection procedure

The basic principle of the new detection strategy is schematically depicted in Fig. 1. The S-DNA complementary to C-RNA serves as a substrate for DNase I and DNA-AgNC/GO nanocomposite is employed as a detection probe to monitor the cleavage reaction promoted by DNase I. The DNA sequence within DNA-AgNC/GO nanocomposite consists of the C-rich template DNA at the 5′-end (purple) and the extension DNA at the 3′-end (yellow) which is complementary to C-RNA. AgNC is first formed around C-rich template DNA and the resulting DNA-AgNC binds to GO through the interaction between the extension DNA and GO.

The procedure in this approach involves DNase I-promoted cleavage reaction of C-RNA/S-DNA hybrid, followed by the incubation of the reaction products with DNA-AgNC/GO nanocomposite. In the absence of DNase I, C-RNA/S-DNA hybrid duplex remains intact and the fluorescence from DNA-AgNC is still significantly quenched by GO because the DNA-AgNC and GO are retained in close proximity. On the contrary, when DNase I is present, S-DNA in C-RNA/S-DNA hybrid duplex is degraded into small DNA fragments, consequently releasing ss C-RNA. The resulting ss C-RNA then forms the duplex with the extension DNA of DNA-AgNC, disrupting its interactions with GO. As a result, the DNA-AgNC is released from GO, leading to significant restoration of the quenched fluorescence of DNA-AgNC. We envisioned that the enhancement of fluorescence signal of DNA-AgNC in response to DNase I activity could be utilized to construct the label-free and fluorescence turn-on assay system for DNase I activity.

3.2. Feasibility of the strategy

Aimed at achieving the efficient assay of DNase I activity, the optimal conditions for DNase I activity assay were first determined by examining the fluorescence intensities of DNA-AgNC at 620 nm, the emission peak wavelength of DNA-AgNC. The results of experiments in which the concentration of GO and the incubation time required to restore the quenched fluorescence from DNA-AgNC were varied, indicate that 36 μg/ml GO and 60 min incubation time are ideal, which are employed for further experiments (Figs. S4 and S5(b)). The stability of the fluorescence from DNA-AgNC was also examined by measuring the fluorescence intensities for 7 days (Fig. S6). The result shows that the fluorescence signals from DNA-AgNC are quite stable for at least 6 days without any significant fluorescence quenching, which ensures the robust utility of DNA-AgNC in this work.

Under the optimal conditions, the fluorescence intensities of DNA-AgNC were measured for different samples to verify the feasibility of this assay strategy. The weak fluorescence signals were obtained from the samples containing S-DNA or DNase I while the sample containing C-RNA showed the high fluorescence signal, indicating that C-RNA complementary to the extension DNA of DNA-AgNC exclusively extracted DNA-AgNC from GO (curve 1, 2, and 3 in Fig. 2). In addition, the sample containing both C-RNA and DNase I exhibited the comparable fluorescence intensity to that from the sample containing C-RNA, which indicates that DNase I did not interfere with the interaction between C-RNA and DNA-AgNC/GO nanocomposite (curve 2 and 4 in Fig. 2). Meanwhile, the low fluorescence intensity was observed in the sample containing both S-DNA and DNase I even though DNase

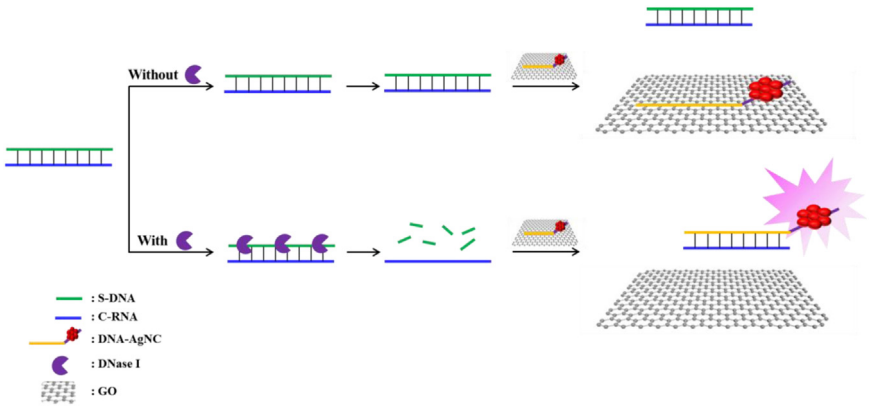

Fig. 1. Schematic illustration of the DNase I activity assay based on DNA-AgNC/GO nanocomposite. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

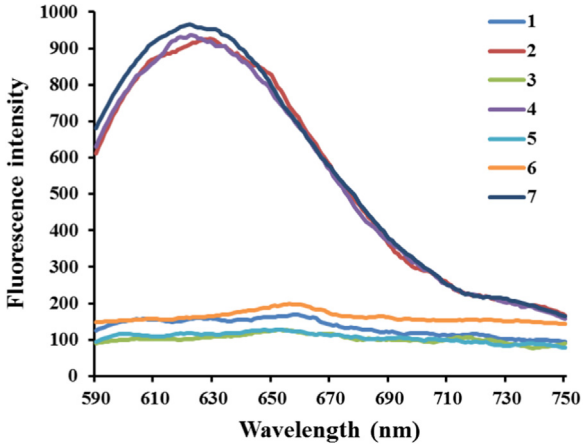

Fig. 2. Feasibility of the DNase I activity assay. Fluorescence emission spectra from DNA-AgNC in the different samples (1: S-DNA, 2: C-RNA, 3: DNase I, 4: C-RNA+DNase I, 5: S-DNA+DNase I, 6: C-RNA/S-DNA hybrid, 7: C-RNA/S-DNA hybrid+DNase I). All the samples contain the DNA-AgNC/GO nanocomposite. The final concentrations of C-RNA, S-DNA, C-RNA/S-DNA hybrid, DNA-AgNC, GO, and DNase I are 1 μM, 1 μM, 1 μM, 500 nM, 36 μg/ml, and 30 U/ml, respectively.

I-catalyzed DNA cleavage reaction took place (curve 5 in Fig. 2). It proves that the DNA cleavage reaction by DNase I could not directly induce the enhancement of fluorescence signal. As expected, the sample containing C-RNA/S-DNA hybrid led to the weak fluorescence emission in the absence of DNase I because C-RNA/S-DNA remained intact and DNA-AgNC was still adsorbed on GO (curve 6 in Fig. 2). Most importantly, an obvious enhancement of fluorescence intensity was observed in the presence of DNase I, indicating that DNase I degraded S-DNA and released C-RNA from C-RNA/S-DNA hybrid duplex, which subsequently extracted DNA-AgNC from GO through the hybridization with the extension DNA (curve 7 in Fig. 2). Additionally, we performed gel electrophoresis to further confirm the detection mechanism of this strategy (Fig. S7). All these results demonstrate that the proposed strategy is quite feasible and could be efficiently employed for the assay of DNase I activity.

3.3. Sensitivity and selectivity of the strategy

The detection sensitivity of this sensing system was next determined by measuring the fluorescence intensities at 620 nm as a function of DNase I concentration (Fig. 3). The results show that the fluorescence intensities increase with increasing concentrations of DNase I up to about 30 U/ml, but reach a plateau at concentrations higher than 30 U/ml (Fig. 3). An excellent linear relationship ($R^2=0.99$) exists in the range of 0–10 U/ml and the limit of detection (LOD) (3σ/slope) is *ca.* 0.10 U/ml (Fig. 3(b)), which is lower than those of the previous fluorescence-based methods (Dou and Yang, 2013; Sun et al., 2013; Zhou et al., 2012).

In order to evaluate the selectivity of the new assay method for DNase I, other enzymes (RNase H, Hind III, Exo I, Exo III, and λ exo) and biological molecules (Cys and ATP) were investigated for their abilities to restore the quenched fluorescence of DNA-AgNC, which were then compared with that of DNase I. As shown in Fig. 4, the remarkable fluorescence enhancement was observed only when the DNase I was applied while other enzymes were not able to restore the fluorescence even at five times higher concentration than that of DNase I. The weak fluorescence intensities were also observed in the presence of other biological molecules such as Cys and ATP. These results clearly confirm that the proposed assay is highly selective for DNase I and the fluorescence of DNA-AgNC is restored exclusively through the DNA cleavage reaction specifically promoted by DNase I.

3.4. Real sample analysis

Finally, a practical applicability of the new assay system was demonstrated by determining the DNase I activity in bovine urine. The urine samples spiked with DNase I were subjected to the assay procedures and DNase I activities of the samples were determined based on the standard curve (Table 1 and Fig. S8). As a result, the reproducibility and precision of this method were quite excellent, yielding coefficients of variation (CV) that are less than 5% and recovery rates of 96.8% and 101.8% (Table 1). These observations suggest that the present method has the great potential to reliably determine the activity of DNase I in real clinical samples.

4. Conclusion

In the study described above, we have developed a new, label-free, and fluorescence turn-on strategy for the sensitive determination of DNase I activity. This system takes advantage of the DNA-AgNC and relies on the unique DNA binding properties and excellent quenching ability of GO. The quenched fluorescence signal

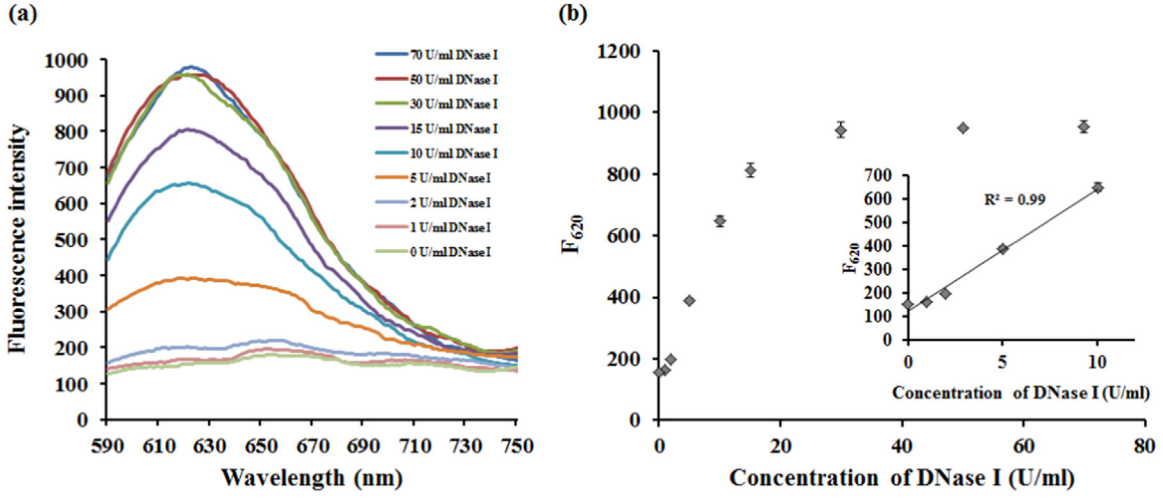

Fig. 3. Sensitivity of the DNase I activity assay. (a) Fluorescence emission spectra from DNA-AgNC after treatment with DNase I at varying concentrations. (b) Fluorescence intensities at 620 nm from DNA-AgNC in the presence of DNase I at varying concentrations. Inset: linear relationship between fluorescence intensity at 620 nm and the DNase I concentration (0–10 U/ml). The final concentrations of C-RNA/S-DNA hybrid, DNA-AgNC, and GO are 1 μM, 500 nM, and 36 μg/ml, respectively.

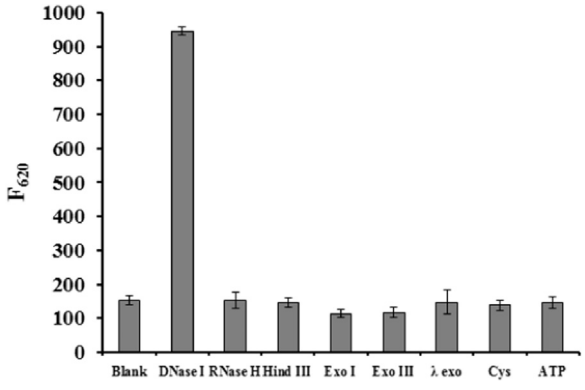

Fig. 4. Selectivity of the DNase I activity assay. Fluorescence responses after treatment with DNase I (30 U/ml), other enzymes at five times higher concentrations (150 U/ml), and other biological molecules (50 μM). The final concentrations of C-RNA/S-DNA hybrid, DNA-AgNC, and GO are 1 μM, 500 nM, and 36 μg/ml, respectively.

Table 1
Determination of DNase I activity in diluted bovine urine.[a]

Added DNase I (U/ml)	Measured DNase I[b] (U/ml)	SD[c]	CV[d] (%)	Recovery[e] (%)
0	Not detectable			
4	4.07	0.17	4.17	101.8
8	7.74	0.09	1.12	96.8

[a] To determine DNase I activity, a calibration curve was first created by using standards having known DNase I activities in bovine urine (Fig. S4). Based on this calibration curve, the fluorescence intensities from the unknown samples were used to determine DNase I activity in bovine urine.
[b] Mean of three measurements.
[c] Standard deviation of three measurements.
[d] Coefficient of variation = SD/mean × 100.
[e] Measured value/added value × 100.

of DNA-AgNC on GO is designed to be recovered very specifically through DNase I-catalyzed DNA cleavage reaction, consequently producing highly enhanced fluorescence signal. Based on this design principle, the DNase I activity was very sensitively determined down to 0.10 U/ml with excellent specificity. In addition, the diagnostic capability of this method has been demonstrated by reliably assaying the DNase I activity in bovine urine. Importantly, the present system overcomes the drawbacks of the conventional methods such as complicated and expensive labeling of organic fluorophores and potential false positive signals although the detection time needs to be further reduced. To the best of our knowledge, this is the first attempt to determine the DNase I activity in a both label-free and fluorescence turn-on manner. Finally, the proposed approach can be generalized to assay other nuclease activities by simply redesigning the substrate probe according to target enzyme, and will pave the way to develop the superior biosensors based on novel nanomaterials.

Acknowledgements

This research was supported by Mid-career Researcher Support Program (No. 2015R1A2A1A01005393) and Bio-Synergy Research Project (No. 2014M3A9C4066457) through the National Research Foundation funded by the Ministry of Science, ICT and Future Planning (MSIP) of Korea. Financial support was also provided by Center for BioNano Health-Guard funded by the MSIP of Korea as Global Frontier Project (Grant H-GUARD_2013M3A6B2078964).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2016.08.073.

References

Arakawa, K., Kawai, Y., Kumamoto, T., Morikawa, N., Yoshida, M., Tada, H., Kawaguchi, R., Taniguchi, K., Miyamori, I., Kominato, Y., 2005. Eur. Heart J. 26, 2375–2380.
Cao, Q., Teng, Y., Yang, X., Wang, J., Wang, E., 2015. Biosens. Bioelectron. 74, 318–321.
Cherepanova, A., Tamkovich, S., Pyshnyi, D., Kharkova, M., Vlassov, V., Laktionov, P., 2007. J. Immunol. Methods 325, 96–103.
Choi, S.-J., Szoka, F.C., 2000. Anal. Biochem. 281, 95–97.
Cui, X., Zhu, L., Wu, J., Hou, Y., Wang, P., Wang, Z., Yang, M., 2015. Biosens. Bioelectron. 63, 506–512.
Dikin, D.A., Stankovich, S., Zimney, E.J., Piner, R.D., Dommett, G.H., Evmenenko, G., Nguyen, S.T., Ruoff, R.S., 2007. Nature 448, 457–460.
Dou, Y., Yang, X., 2013. Anal. Chim. Acta 784, 53–58.
Economidou-Karaoglou, A., Lans, M., Taper, H., Michaux, J.-L., Roberfroid, M., 1988. Cancer 61, 1838–1843.
Fujihara, J., Tabuchi, M., Inoue, T., Yasuda, T., Fujita, Y., Takeshita, H., 2011. Anal. Biochem. 413, 78–79.
He, S., Song, B., Li, D., Zhu, C., Qi, W., Wen, Y., Wang, L., Song, S., Fang, H., Fan, C., 2010. Adv. Funct. Mater. 20, 453–459.
Huang, J., Gao, X., Jia, J., Kim, J.-K., Li, Z., 2014. Anal. Chem 86, 3209–3215.
Hummers Jr, W.S., Offeman, R.E., 1958. J. Am. Chem. Soc. 80, 1339–1339.
Jang, H., Ryoo, S.R., Kim, Y.K., Yoon, S., Kim, H., Han, S.W., Choi, B.S., Kim, D.E., Min, D.H., 2013. Angew. Chem. Int. Ed. 52, 2340–2344.
Kawai, Y., Yoshida, M., Arakawa, K., Kumamoto, T., Morikawa, N., Masamura, K., Tada, H., Ito, S., Hoshizaki, H., Oshima, S., 2004. Circulation 109, 2398–2400.
Lee, T.H., Montalvo, L., Chrebtow, V., Busch, M.P., 2001. Transfusion 41, 276–282.
Ling, K., Jiang, H., Li, Y., Tao, X., Qiu, C., Li, F.-R., 2016. Biosens. Bioelectron. 86, 8–13.
Liu, X., Wang, F., Aizen, R., Yehezkeli, O., Willner, I., 2013. J. Am. Chem. Soc. 135, 11832–11839.
Liu, Z., Robinson, J.T., Sun, X., Dai, H., 2008. J. Am. Chem. Soc. 130, 10876–10877.
Mohanty, N., Berry, V., 2008. Nano Lett. 8, 4469–4476.
Morikawa, N., Kawai, Y., Arakawa, K., Kumamoto, T., Miyamori, I., Akao, H., Kitayama, M., Kajinami, K., Lee, J.-D., Takeshita, H., 2007. Eur. Heart J. 28, 2992–2997.
Nadano, D., Yasuda, T., Kishi, K., 1993. Clin. Chem. 39, 448–452.
Nakajima, T., Takagi, R., Tajima, Y., Makita, C., Kominato, Y., Kuribara, J., Ohshima, S., Tada, H., Tsurugaya, H., Kobayashi, Y., 2009. Clin. Chim. Acta 403, 219–222.
Napirei, M., Karsunky, H., Zevnik, B., Stephan, H., Möröy, T., 2000. Nat. Genet. 25, 177–181.
Park, K.S., Kim, M.I., Woo, M.-A., Park, H.G., 2013. Biosens. Bioelectron. 45, 65–69.
Park, K.S., Lee, C.Y., Park, H.G., 2014a. Analyst 139, 4691–4695.
Park, K.S., Oh, S.S., Soh, H.T., Park, H.G., 2014b. Nanoscale 6, 9977–9982.
Richards, C.I., Choi, S., Hsiang, J.-C., Antoku, Y., Vosch, T., Bongiorno, A., Tzeng, Y.-L., Dickson, R.M., 2008. J. Am. Chem. Soc. 130, 5038–5039.
Sato, S., Fujita, K., Kanazawa, M., Mukumoto, K., Ohtsuka, K., Takenaka, S., 2009. Anal. Chim. Acta 645, 30–35.
Spandidos, D., Ramandanis, G., Garas, J., Kottaridis, S., 1980. Eur. J. Cancer 16, 1615–1619.
Sun, S.-K., Wang, B.-B., Yan, X.-P., 2013. Analyst 138, 2592–2597.
Tamkovich, S.N., Cherepanova, A.V., Kolesnikova, E.V., Rykova, E.Y., Pyshnyi, D.V., Vlassov, V.V., Laktionov, P.P., 2006. Ann. N. Y. Acad. Sci. 1075, 191–196.
Varghese, N., Mogera, U., Govindaraj, A., Das, A., Maiti, P.K., Sood, A.K., Rao, C., 2009. ChemPhysChem 10, 206–210.
Vosch, T., Antoku, Y., Hsiang, J.-C., Richards, C.I., Gonzalez, J.I., Dickson, R.M., 2007. Proc. Natl. Acad. Sci. USA 104, 12616–12621.
Wu, Z., Wu, Z.-K., Tang, H., Tang, L.-J., Jiang, J.-H., 2013. Anal. Chem. 85, 4376–4383.
Xu, X., Han, M.S., Mirkin, C.A., 2007. Angew. Chem. Int. Ed. 119, 3538–3540.
Yeh, H.-C., Sharma, J., Han, J.J., Martinez, J.S., Werner, J.H., 2010. Nano Lett. 10, 3106–3110.
Zhang, H., Zhang, H., Aldalbahi, A., Zuo, X., Fan, C., Mi, X., 2016a. Biosens. Bioelectron. . http://dx.doi.org/10.1016/j.bios.2016.07.030
Zhang, K., Wang, K., Zhu, X., Xie, M., 2016b. Biosens. Bioelectron. 78, 154–159.
Zhang, Y., Zhu, C., Zhang, L., Tan, C., Yang, J., Chen, B., Wang, L., Zhang, H., 2015. Small 11, 1385–1389.
Zhou, Z., Zhu, C., Ren, J., Dong, S., 2012. Anal. Chim. Acta 740, 88–92.
Zhu, B., Gong, Y., Chen, P., Zhang, H., Zhao, T., Li, P., 2014. Mol. Cell. Biochem. 393, 23–32.