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Graphene Oxide-based Direct Measurement of DNase I Activity with Single Stranded DNA

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Recent studies have shown that single-stranded DNA adsorbed onto graphene oxide is protected from DNase I cleavage. However, double-stranded DNA bound to graphene oxide and could be digested by DNase I. To elucidate whether single-stranded DNA is protect from DNase I in the presence of graphene oxide, this study conducted DNase I digestion using single-stranded DNA and single-stranded DNA containing the duplex region in the presence of graphene oxide. Addition of DNase I resulted in restoration of the fluorescence emission that had been quenched when DNA was adsorbed to graphene oxide. It indicates that DNase I cleaved the adsorbed single-stranded DNA onto graphene oxide, which was sufficient for the detection of DNase I activity.

Key Words : Graphene oxide, Platform, Biosensor, DNase I

Introduction

Graphene oxide (GO) is a one-atom thick with each carbon covalently bonded through sp^2 hybridization, resulting in a trigonal planar structure. GO contains hydroxyl, carboxyl, and epoxy groups on its surface or edge, and has a high surface area onto which nucleobases of DNA can be adsorbed through hydrophobic and π-stacking interactions.[1-3] Recently, GO has attracted as a biosensor,[4-6] and platform[7-9] for detecting biomolecules or cells with high sensitivity and selectivity.

A 6-fluorescein amidite (FAM)-labeled single-stranded DNA (ssDNA) was adsorbed onto GO, resulting in rapid quenching of the fluorescence signal.[7] However, the fluorescence emission of ssDNA was restored through duplex DNA formation with complementary HIV1 DNA.[7] Detection of the sensitivity and efficiency of target DNA was dramatically increased by the use of a dye-labeled probe through exonuclease III-aided signal amplification in the presence of GO.[10,11] In addition, GO enabled differentiation between wild-type and single- base-mismatched sequences of molecular beacon based on fluorescence emission changes.[12,13] To detect duplex DNA unwinding by helicase, unwinding of FAM-labeled double-stranded DNA (dsDNA) led to quenching of the fluorescence emission after adsorption of ssDNA onto GO depending on the helicase concentration.[8]

Recent studies[14,15] have reported that adsorbed ssDNA or dsDNA was protected from DNase I cleavage. By contrast, DNase I digested the adsorbed dsDNA onto GO and showed sufficient sensitivity for the detection of enzyme activity.[16] Moreover, human thrombin protein could recognize its aptamer DNA, which was adsorbed onto GO, resulting in the release of the aptamer DNA-protein complex from GO.[7] Recent publications[7,14-16] demonstrated that enzymes were able to recognize and digest the DNA adsorbed onto GO.

To elucidate whether single-stranded DNA is protected from DNase I in the presence of graphene oxide, this study conducted DNase I digestion using ssDNA and ssDNA containing the duplex region in the presence of graphene oxide.

DNase I degrades ssDNA or dsDNA into single nucleotide and oligonucleotides with 5'-phospho and 3'-hydroxy termini. DNase I is extensively utilized in DNase I footprinting,[17] removal of genomic DNA in cell extract purification,[18] genetic identification for forensic purposes,[19] and plasmid for protein excision from *in vitro*-transcribed RNA.[20]

The results of this study showed that DNase I digested ssDNA and ssDNA containing the duplex region that had been adsorbed onto GO, which was sufficient for the detection of DNase I activity.

Materials and Methods

GO (2 mg/mL) was purchased from Nanocs (New York, USA). DNase I (5.0 U/μL) was obtained from TakaRa (Shiga, Japan) and oligomers were synthesized and purified by polyacrylamide gel electrophoresis (Sigma-Aldrich, St. Louis, MO, USA). All chemicals were of analytical grade.

GO Measurement. Raman spectroscopy measurement was carried out at room temperature with Monora500i Raman spectrometer (Dongwoo Optron, Gwangju, South Korea), with excitation provided by a 633 nm He-Ne laser line. The size and zeta potential of GO was determined using ZetaPALS (Brookhaven Instrument, New York, USA). The analysis was performed at 25 °C using Milli-Q water or reaction buffer. The zeta-potential was average of three independent measurements.

Fluorescence Measurement. The DNase I (5.0 U/μL) reaction was conducted using 2 mg/mL of GO and 30 nM of fluorescein or FAM-labeled ssDNA in reaction buffer (10 mM Tris, pH 7.5, 5 mM $MgCl_2$, 7.5 mM dithiothreitol) at room temperature (~20 °C). Fluorescence quenching was monitored

in the presence of fluorescent ssDNA by adding GO to the reaction. Fluorescence restoration was measured at regular intervals for 1 h by adding DNase I to the reaction. Fluorescence emission was measured using an RF 5301PC system (Shimadzu, Kyoto, Japan) at excitation wavelengths of 485 nm and 488 nm for fluorescein and FAM-labeled DNA, respectively, and emission wavelengths from 502-600 nm. DNase I was added at a concentration of 0.02 U/μL for the digestion reaction.

Gel Electrophoresis of DNase I Cleavage. As mentioned in fluorescence emission assay, reactions were conducted with 0.025 U/μL of DNase I at room temperature (approximately 20 °C) in the presence of ssDNA 1 and GO. The gel was run at 80 volt for 40 min on 12 % native polyacrylamide gel and dried for visualization with Kodak Image scanner at 470 nm excitation and 530 nm emission wavelengths.

Results and Discussion

Adsorption of ssDNA to GO. According to manufactured company of GO, it was reported that GO contains 1.1 nm thickness and single atomic layer over 85%. Raman spectra showed D peaks ~1350 cm^{-1} and G peaks ~1590 cm^{-1}, confirming the lattice distortion of GO structure (data not shown).[21] Dynamic light scattering measured that mean effective diameters of GO are 435 nm and 10.6 μm in pure water and reaction buffer, respectively. The surface potential of GO is −26.8 mV in pure water. In reaction buffer, zeta potential of GO is −9.87 mV, indicating that more molecules like DNA or proteins binding occurred to the surface of GO.

Figure 1 shows the schematic representation of the fluorescence assay of DNase I in the presence of GO. The first row shows GO-based ssDNA adsorption and ssDNA digestion by DNase I. The second row represents GO-based DNA cleavage of ssDNA containing the duplex region. DNA adsorption and cleavage were monitored by fluorescence quenching and restoration.

Strong fluorescence emission was observed due to the fluorescein or fluorescein-amidite-attached DNAs (curve a in Figure 2). Addition of GO to ssDNA 1 or ssDNA 2 resulted in 98% quenching of fluorescence emission in 10 min in comparison with the value of ssDNA 1 (Figure 2(a)) or ssDNA 2 (data not shown). Based on fluorescence quenching, ssDNAs were adsorbed with high efficiency relative to ssDNAs containing the duplex region (Figure 2(b) and 3(b)). This indicates that the duplex region of the ssDNA was not adsorbed strongly to the surface of GO because of the excluded nucleobases in the duplex region. Dye-tagged ssDNA was adsorbed strongly to the surface of GO through the noncovalent interaction between GO and ssDNA, resulting in highly efficient fluorescence quenching.[7,9] In

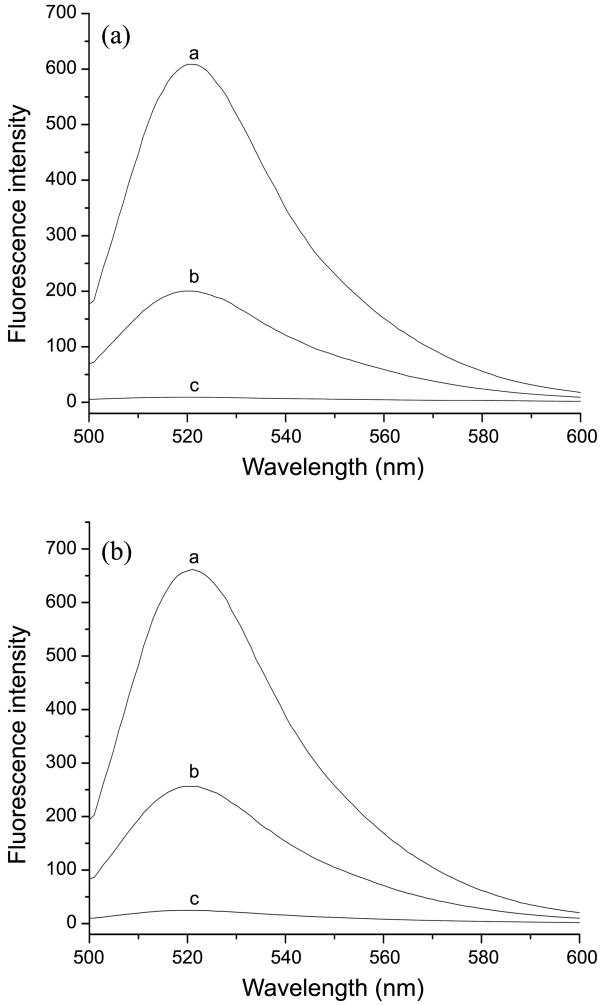

Figure 2. Fluorescence emission spectra of ssDNA 1 (a) and ssDNA 1-ssDNA 3 (b). Curve a shows the fluorescence emission of DNA alone. Curve b and c represent the fluorescence emissions of DNA with GO either in the presence or absence of DNase I, respectively.

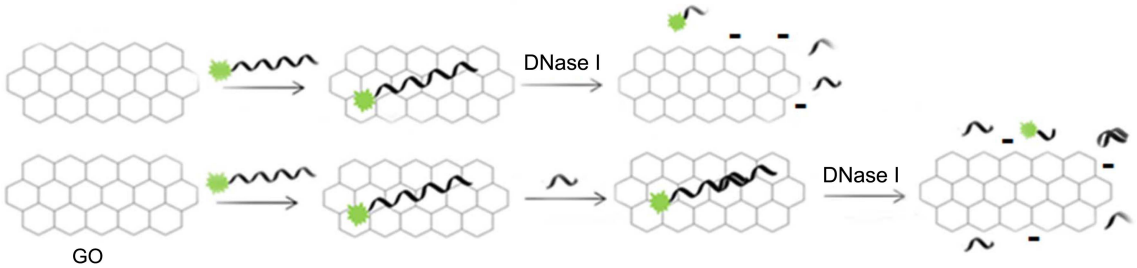

Figure 1. Schematic representation for the detection of DNase I activity in the presence of GO. The first row shows GO-based ssDNA adsorption and DNA digestion by DNase I. The second row indicates GO-based DNA cleavage for ssDNA 1 containing the duplex DNA which is adsorbed onto GO.

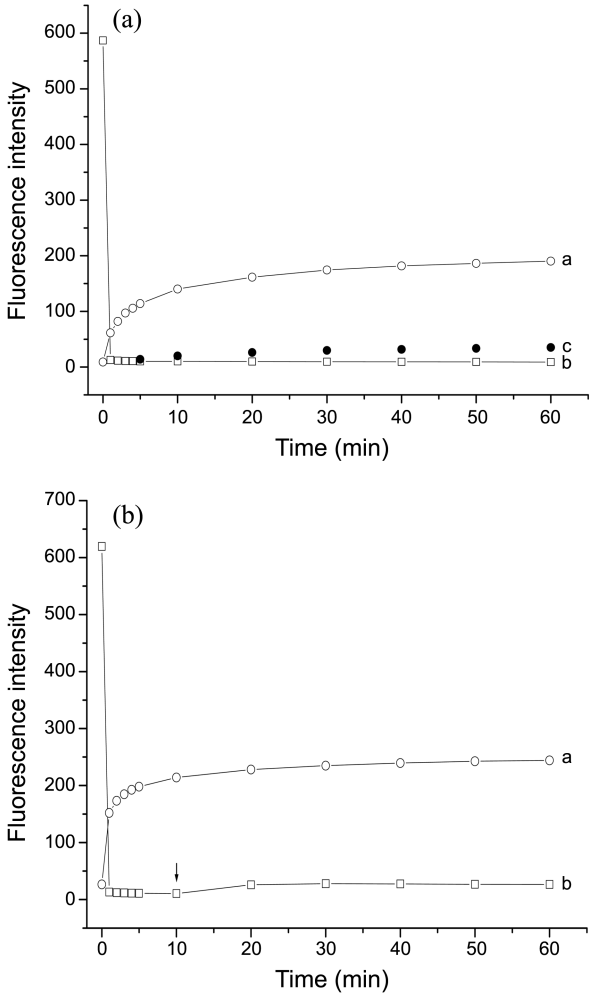

Figure 3. Fluorescence quenching and restoration kinetics of ssDNA 1 (a) and ssDNA1-ssDNA 3 (b) as a function of time. Curve a shows fluorescence quenching of DNA in the presence of GO. Curve b represents fluorescence restoration of the adsorbed DNA by DNase I. Curve c (Figure 3(a)) shows fluorescence change for the release of adsorbed DNA from GO by adding BSA. Arrow indicates a time when ssDNA 3 was added to ssDNA 1 reaction.

addition, Wu *et al*.[21] reported that ssDNAs were adsorbed more efficiently than dsDNA to the surface of GO.

Detection Sensitivity of DNase I Activity. Digested dsDNA and ssDNA products were not adsorbed to GO because there were not a sufficient number of bases to be able to interact with the surface of GO.[7,9] Thus, fluorescence emissions were restored in proportion to the amount of DNase I-digested products. To observe the real-time adsorption and desorption between DNA and GO, fluorescence emission was measured as a function of incubation time (Figure 3). As shown above, adsorption of the ssDNA 1 reached equilibrium within 10 min at room temperature (~20 °C). By contrast, enzymatic digestion showed that the fluorescence emission of ssDNA 1 was restored to 24% 10 min after DNase I addition (Figure 3(a)). Thus, the digestion reaction was not sufficiently fast for comparison with the adsorption of DNA to GO.

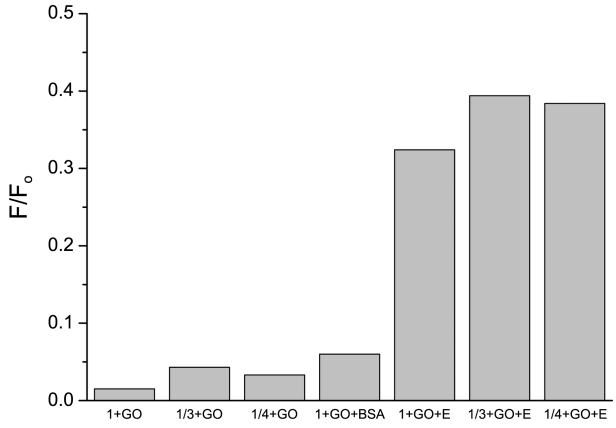

Figure 4. F/F_0 was plotted against reaction conditions with DNA. 1, 1/3, and 1/4 represent ssDNA 1, ssDNA 1-ssDNA 3, and ssDNA 1-ssDNA 4, respectively. E and BSA indicate the addition of DNase I enzyme and bovine serum albumin to reactions, respectively.

To analyze the kinetic results, F/F_0 versus reaction conditions was plotted as shown in Figure 4. F_0 and F represent the fluorescence intensities of DNA either in the absence or presence of reactants, respectively. For ssDNA 1 digestion, quenched fluorescence was restored to approximately 33% of the value observed for the ssDNA 1 alone. By contrast, fluorescence restoration was approximately 40% for ssDNA 1-ssDNA 3 or ssDNA 1-ssDNA 4. It showed that fluorescence restoration by DNase I was enhanced for the ssDNA 1 containing the duplex region in comparison to that of ssDNA. It demonstrated that DNase I degraded the ssDNA 1containing the duplex region more efficiently than ssDNA 1 in that the duplex regions of ssDNA 1-ssDNA 3 or 4 were not tightly adsorbed onto the surface of GO relative to that of ssDNA 1. As reported in recent publications,[7,15,16,22] ssDNA was adsorbed more strongly onto the surface of GO than dsDNA due to the interaction between nucleobases of DNA and hexagonal rings of GO.

To measure whether addition of enzyme to ssDNA-GO complex forces the adsorbed ssDNA off of GO by the interaction of enzyme and GO, fluorescence change was monitored in the presence of bovine serum albumin (BSA) to ssDNA 1-GO complex (Figure 2(a) and Figure 4). Addition of BSA led to an increase of fluorescence intensity by 30 for 6.96 μg of BSA (corresponding to 20 U of DNase I used in this study) compared to that of the ssDNA 1-GO complex. This indicates that adsorbed ssDNA was not largely released from GO by the addition of enzyme (curve c in Figure 2(a)).

It was further investigated with the signal to background (S/B) ratio to estimate fluorescence restoration efficiency by enzyme reaction. The S/B ratio was defined as $(F_{DNA\text{-}GO/enzyme} - F_{buffer}) / (F_{DNA\text{-}GO} - F_{buffer})$, where $F_{DNA\text{-}GO/enzyme}$, $F_{DNA\text{-}GO}$, and F_{buffer} represent the fluorescence intensities for the cleavage of DNA from the DNA-GO complex, adsorption of DNA to GO, and reaction buffer, respectively.[18,19] The S/B ratio was estimated to be 22.3 and 12.0 for ssDNA 1 and ssDNA 1 containing the duplex region, respectively. The S/B ratio was

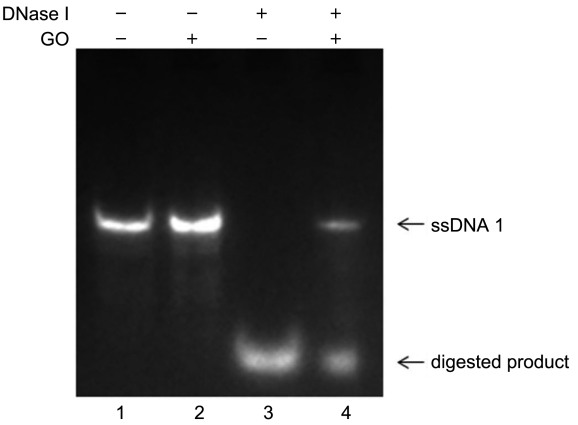

Figure 5. Images of gel electrophoresis of DNase I reaction. Lanes 1 and 2 contain ssDNA 1 either in the absence or presence of GO, respectively. Lane 3 and 4 represent the DNase I digestion of ssDNA 1 either in the absence or presence of DNase I (0.025 U/μL), respectively. The top and bottom arrows indicate the undigested ssDNA 1 and DNase I- digested product, respectively.

Table 1. Sequences of the oligonucleotides used in this study

Type	Sequences
ssDNA 1	5'-Flc-CAT GCC TGC AGG TGG ACT CTC GAG GAT CCC CGG GTA CCG AAA AAA AAA A-3'
ssDNA 2	5'-6FAM-AAA AAA AAA ACG GTA CCC GGG GAT CCT CGA GAG TCC ACC TGC AGG CAT G-3'
ssDNA 3	5'-TAC CCG GGG ATC CTC GAG-3'
ssDNA 4	5'-ATC CTC GAG-3'

Flc and FAM indicate fluorescein and 6-fluorescein amidite dyes, respectively.

low for ssDNA containing the duplex region, because $F_{DNA\text{-}GO}$ of ssDNA 1 containing the duplex region was large relative to the value of ssDNA 1 (Figure 2(b) and 3(b)).

In addition to fluorescence emission measurement of GO-based DNase I cleavage, Gel electrophoresis reactions were conducted with 0.025 U/μL of DNase I in the presence of ssDNA 1 and GO. Gel was run at 80 volt for 40 min on 12% native polyacrylamide gel and dried for visualization with Kodak Image scanner at 470 nm excitation and 530 nm emission wavelengths. Figure 5 shows that ssDNA 1 was completely digested in the absence of GO by DNase I (lane 3). In the presence of GO, however some of the ssDNA 1 was degraded by 0.025 U/μL of DNase I (lane 4). Gel electrophoresis revealed an undigested ssDNA 1 band at the top of the gel (indicated with arrow) and a degraded DNA band at the bottom of the gel (indicated with arrow) in the presence of GO. This indicates that DNase I digested the adsorbed ssDNA, but did not cleave all of the adsorbed ssDNAs in the presence of GO. This result is consistent with the results of the fluorescence assay by DNase I.

In addition, Gel shift assay was conducted to detect the ssDNA-GO complex with the various concentrations of GO (data not shown). But the ssDNA 1-GO complex was not shown on polyacrylamide gel due to the size of GO particles in reaction buffer (10.6 μm diameter). It has been reported previously that dsDNA-GO complex was blocked in agarose gel and dwelled in the sample well.[20,22]

According to results from this study, ssDNA was not fully protected from DNase I cleavage and the GO-based fluorescence assay showed the high fluorescence restoration intensity enough to detect DNase I activity.

Conclusions

Upon the adsorption of DNA to GO, ssDNA was adsorbed more efficiently than ssDNA containing the duplex region. According to results from this study, ssDNA was not fully protected from DNase I cleavage and the GO-based fluorescence assay showed the high fluorescence restoration intensity enough to detect DNase I activity.

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