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On-chip graphene oxide aptasensor for multiple protein detection

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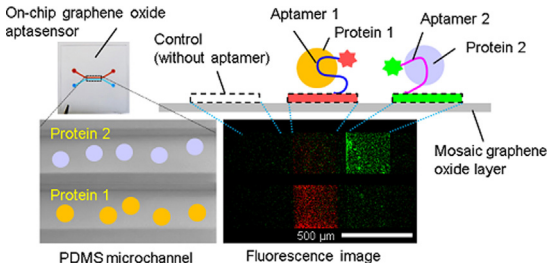
HIGHLIGHTS

- On-chip aptasensor built on graphene oxide surface fixed on solid support.
- Multichannel configuration realizes simultaneous detection of 3–5 samples.
- Versatility of graphene oxide aptasensor formed on solid surface was studied.
- DNA and RNA aptamers immobilized on graphene oxide surface maintain their activity.
- Simultaneous multiple protein detection was demonstrated.

GRAPHICAL ABSTRACT



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ABSTRACT

The versatility of an on-chip graphene oxide (GO) aptasensor was successfully confirmed by the detection of three different proteins, namely, thrombin (TB), prostate specific antigen (PSA), and hemagglutinin (HA), simply by changing the aptamers but with the sensor composition remaining the same. The results indicate that both DNA and RNA aptamers immobilized on the GO surface are sufficiently active to realize an on-chip aptasensor. Molecular selectivity and concentration dependence were investigated in relation to TB and PSA detection by using a dual, triple, and quintuple microchannel configuration. The multiple target detection of TB and PSA on a single chip was also demonstrated by using a 2×3 linear-array GO aptasensor. This work enables us to apply this sensor to the development of a multicomponent analysis system for a wide variety of targets by choosing appropriate aptamers.

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Abbreviations: GO, graphene oxide; TB, thrombin; PSA, prostate specific antigen; HA, hemagglutinin; TBA, thrombin binding aptamer; PSAA, prostate specific antigen binding aptamer; HAA, hemagglutinin binding aptamer.

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1. Introduction

Graphene is a two-dimensional sp^2 carbon sheet with atomic layer thickness [1,2]. First, graphene was isolated, and it attracted attention owing to its unique structure and properties. Now graphene oxide (GO) has started to generate considerable interest. GO is an oxidized form of graphene and it also has an atomically thin sheet-like structure, which contains nanometer-sized graphene-like sp^2 domains [3–5]. Of several useful properties of GO, an important one for the present study is that it is an excellent acceptor for fluorescence resonance energy transfer (FRET) in the entire visible wavelength region. This makes GO a promising material for a FRET-based biosensor. In addition, unlike graphene, GO is water dispersible and readily synthesized on a large scale [6,7]. Another useful property is its strong molecular adsorption via a π–π interaction. Thus, GO exhibits a high affinity to aptamers, which are selected single-strand oligonucleotides that bind to specific targets [8,9]. Aptamers offer many advantages as a molecular recognition probe. They have wide variety of targets, can be flexibly designed without loss of activity, and they are easy and cheap to produce. There are DNA and RNA-type aptamers, which we can use to suit our purpose. DNA is more stable and easier to mass-produce than RNA. On the other hand, RNA is flexible in structure and thus an RNA aptamer can have wider variety of targets than a DNA aptamer [10]. All the above features make GO suitable for biological applications.

By using a GO and dye-conjugated aptamer, we can realize a unique type of FRET biosensor. By using GO, the selective biological response of the aptamer can be converted to a fluorescence signal, which is a measurable physical quantity. In detail, the GO aptasensor detection process is as follows. At the initial stage, when the dye-conjugated aptamer is adsorbed on the GO surface via π–π interactions, the dye is located close to the GO surface and its fluorescence is well quenched by GO and is barely observable. If a target is present in the sample, the aptamer forms a complex with the target and leaves the GO surface. At the same time, the dye molecule also leaves the GO surface and the fluorescence of the dye recovers. By employing this concept, early studies on this type of sensor have demonstrated DNA sensors (i.e., sensors for complementary DNA) [11–16] and aptasensors in an aqueous dispersion of GO [17–21].

In contrast, we have proposed and developed a protein detection system that works on a GO layer supported on a solid surface [22]. In our system, the aptamer terminus opposite the dye-labeled end is firmly fixed to the GO surface by a pyrene linker molecule that shows a strong affinity to the sp^2 domains in the GO [5,23,24]. Thus, the aptamer stays close to the GO surface after forming an aptamer/protein complex. The system allows us to undertake molecular detection on a solid surface, unlike with conventional GO aptasensors which operate in a solution. This is a useful point for realizing an on-chip sensor.

Based on this system, we have recently demonstrated the first example of a linear-array GO aptasensor by forming a linear array of several different aptamers on the surface of a GO layer and implementing a microfluidic device across the array [25]. A linear-array GO aptasensor is a smart approach for comparing the responses of different aptamers on a single chip simultaneously. It is also advantageous that the on-chip sensor requires 1 μL or less of solution, and no out-of-chip sample pretreatment, such as labeling or the mixing of liquids. The recognition process is complete within 2 min of adding the sample solution.

In general, aptasensors are versatile because the sensors can be extended to the detection of many different targets by replacing the aptamers. Although we have already demonstrated that this approach works for thrombin detection, we have not yet confirmed whether the versatility is valid for an aptamer immobilized on a solid surface. Thus, in this work, we investigated the extension of our GO FRET aptasensor to other types of DNA and RNA aptamers. We also increased the number of microchannels, which allowed us to obtain a consistent reference-to-sample comparison, by implementing a dual, triple, and quintuple channel microfluidic device on a single chip. If we use one channel as a reference, it can be employed as an internal standard for eliminating the effect of fluorescence degradation caused by laser exposure and other noises. The precise reference comparison allows us to confirm molecular selectivity and the concentration dependence of the fluorescence intensity in relation to thrombin (TB) and prostate specific antigen (PSA) detection by using TB- and PSA-binding DNA aptamers (TBA and PSAA), respectively. We also fabricated the GO aptasensor by using an RNA aptamer, namely hemagglutinin (HA)-binding RNA aptamer (HAA). We found that both DNA and RNA aptamers immobilized on a GO surface maintained the biological activity needed to realize an on-chip aptasensor. Based on these findings, we demonstrated the multiple target detection of TB and PSA on a single chip by using a 2×3 linear-array GO aptasensor.

2. Experimental

2.1. Materials

The DNA and RNA aptamers were purchased from Sigma Genosys. The 5′- and 3′-termini of the aptamer sequences were modified with green fluorescence 6-carboxyfluorescein (FAM) and an amine group. The aptamer sequences were as follows: TBA (5′-GGTTGGTGTGGTTGG-3′) [26], PSAA (5′-TTTAATTAAAGCTCTC-CATCAAATAGCTTTTTTTTTT-3′) [27], and HAA (5′-GUCGGCA-UGCGGUA-3′) [28]. Another TB-binding DNA aptamer, TBA29 (5′-AGTCCGTGGTAGGGCAGGTTGGGGTGACT-3′) [29] was modified with red fluorescence TAMRA instead of FAM. The DNA and RNA aptamers were dissolved in 100 mM phosphate buffer solution and DNase/RNase-free DI water, respectively, and prepared as 100 μM solutions. Phosphate buffer (pH 7.4) and RNase-free DI water (UltraPure™ DNase/RNase-Free Distilled Water) were purchased from Nacalai Tesque Inc. and Life Technologies, respectively. 1-Pyrenebutanoic acid–succinimidyl ester was purchased from Invitrogen. *N*,*N*-Dimethylformamide (DMF) was obtained from Kanto Chemical Co., Inc. Alpha-human thrombin (ICN Biochemicals), alpha-human albumin (ICN Biochemicals), and PSA from human semen (Sigma–Aldrich), were dissolved in DI water. Influenza A H3N2 (A/Aichi/2/1968) Hemagglutinin protein (Sino Biological Inc.) was dissolved in DNase/RNase-free DI water. DI water (Millipore, >18 MΩ cm) and DNase/RNase-free DI water, respectively, were used in all the aqueous solution preparation and washing procedures in the DNA and RNA aptamer experiments.

2.2. Apparatus and measurement conditions

An Olympus BX51-FV300 confocal laser scanning microscope (LSM) was used to obtain fluorescence images. We used a 505–525 nm band-pass filter and a 565 nm high-pass filter for the fluorescence observations of FAM (λ_{max}(abs)/λ_{max}(em) = 494 nm/518 nm) and TAMRA (λ_{max}(abs)/λ_{max}(em) = 555 nm/580 nm), respectively, with a 488 nm laser light source. The fluorescence images were obtained through a glass plate with an objective lens (UPlan Apo 10× LSM or a water-immersion objective lens Plan Apo 40× WLSM (Olympus)) within about 2 min of injecting a certain concentration of protein solution and reference water into the sample and the reference channels, respectively. The reaction time was sufficient for the aptamer to complete protein recognition [22]. The required sample volumes for each channel were smaller than 1 μL. Atomic force microscope (AFM) images were recorded in the AC (tapping) mode under atmospheric conditions using a

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