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Rapid and simple G-quadruplex DNA aptasensor with guanine chemiluminescence detection

Sandy Cho [a,1], Lucienne Park [a,b,1], Richard Chong [a], Young Teck Kim [c], Ji Hoon Lee [a,*]

[a] Luminescent MD, LLC, 20140 Scholar Dr., Hagerstown, MD 21742, United States
[b] Department of Biology, University of Maryland, College Park MD 20742, United States
[c] Department of Wood Science & Forest Products, Virginia Polytechnic Institute & State, University, Blacksburg, VA 24061, United States

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ABSTRACT

Cost-effective and sensitive aptasensor with guanine chemiluminescence detection capable of simply quantifying thrombin in human serum was developed using thrombin aptamer (TBA), one of the G-quadruplex DNA aptamers, without expensive nanoparticles and complicated procedures. Guanines of G-quadruplex TBA-conjugated carboxyfluorescein (6-FAM) bound with thrombin do not react with 3,4,5-trimethoxylphenylglyoxal (TMPG) in the presence of tetra-*n*-propylammonium hydroxide (TPA), whereas guanines of free TBA- and TBA-conjugated 6-FAM immobilized on the surface of graphene oxide rapidly react with TMPG to emit light. Thus, guanine chemiluminescence in 5% human serum with thrombin was lower than that without thrombin when TBA-conjugated 6-FAM was added in two samples and incubated for 20 min. In other words, the brightness of guanine chemiluminescence was quenched due to the formation of G-quadruplex TBA-conjugated 6-FAM bound with thrombin in a sample. High-energy intermediate, capable of emitting dim light by itself, formed from the reaction between guanines of TBA and TMPG in the presence of TPA, transfers energy to 6-FAM to emit bright light based on the principle of chemiluminescence energy transfer (CRET). G-quadruplex TBA aptasensor devised using the rapid interaction between TBA-conjugated 6-FAM and thrombin quantified trace levels of thrombin without complicated procedures. The limit of detection (LOD = background + 3 × standard deviation) of G-quadruplex TBA aptasensor with good linear calibration curve, accuracy, precision, and recovery was as low as 12.3 nM in 5% human serum. Using the technology reported in this research, we expect that various types of G-quadruplex DNA aptasensors capable of specifically sensing a target molecule such as ATP, HIV, ochratoxin, potassium ions, and thrombin can be developed.

1. Introduction

Since 1990 (Ellington and Szostak, 1990; Tuerk and Gold, 1990), it has been well-known that nucleic acid aptamers selected from a large pool of random sequences, instead of expensive antibodies produced from the sacrifice of small animals, can bind specifically to target molecules in a sample collected from human (Bock et al., 1992; Phan et al., 2005), food (Cruz-Aguado and Penner, 2008), and nature (Ueyama et al., 2002). For example, 15 nt thrombin aptamer (TBA, 5′-GGTTGGTGTGGTTGG-3′) was developed to inhibit thrombin activity capable of converting fibrinogen into clottable fibrin in the hemostasis process (Bock et al., 1992). Studies with circular dichroism (CD) spectra (Pasternak et al., 2011), NMR (Kelly et al., 1996), and X-ray (Kelly et al., 1996; Padmanabhan et al., 1993) were confirmed that the shape of TBA bound with two anion binding recognition exosites of thrombin is G-quadruplex as shown in Fig. 1(a). G-quadruplex was formed with two square planar structures. The square planar structure called a guanine tetrad was formed through hydrogen bonding between four guanines as shown in Fig. 1(b). A number of anti-parallel or parallel G-quadruplex aptamers were developed to bind and quantify a wide variety of target molecules such as ATP (Huizenga and Szostak, 1995), hemin (Travascio et al., 1998), HIVs (Phan et al., 2005), insulin (Yoshida et al., 2009), ochratoxin (Cruz-Aguado and Penner, 2008), potassium ions (Ueyama et al., 2002), Sars-CoV helicase (Shum and Tanner, 2008), and selerostin (Shum et al., 2011) in a sample. Using the characteristics of G-quadruplex DNA aptamers, currently, various types of aptasensors with chemiluminescence (Bi et al., 2010; Freeman et al., 2011; Freeman et al., 2012; Zhou et al., 2012), colorimeter (Zhang et al., 2011), electrochemical (Yuan et al., 2011), or fluorescence (Yuanboonlim et al., 2012) detection have been developed.

Glow chemiluminescence with the reaction between guanine nucleotides and 3,4,5-trimethoxylphenylglyoxal (TMPG) was observed

* Corresponding author. Tel.: +1 301 393 9091.
E-mail address: jhlee@luminescentmd.com (J.H. Lee).
[1] These authors contributed equally in this research.

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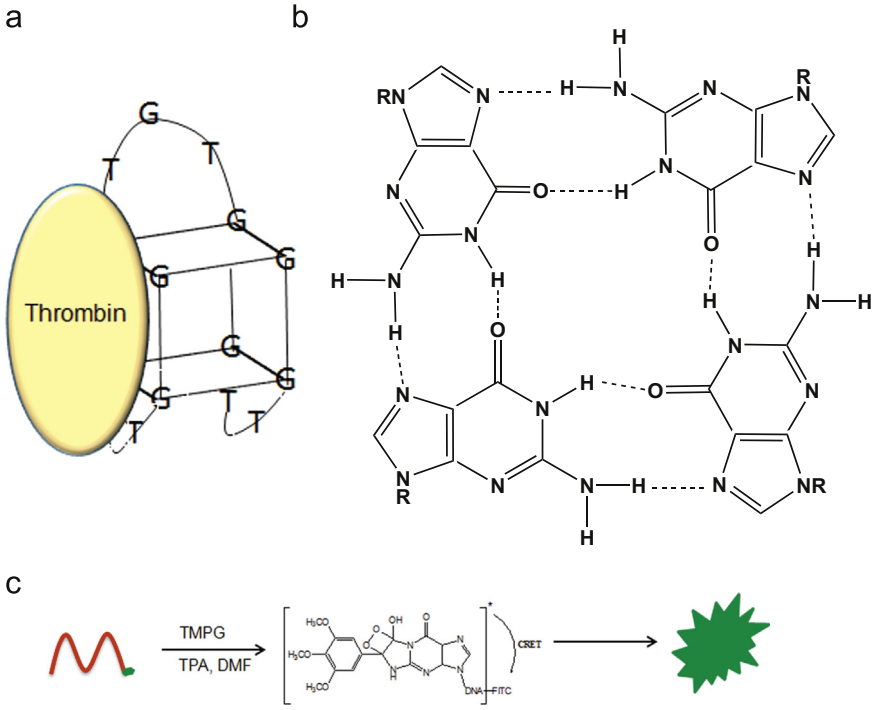

Fig. 1. (a) G-quadruplex TBA aptamer bound with thrombin, (b) G-tetrad structure of guanines in G-quadruplex TBA, and (c) CRET between high-energy intermediate and FITC in guanine chemiluminescence.

(Kai et al., 1999). Recently, Yamasuji et al. (2011) observed bright chemiluminescence emitted in the presence of fluorescein-5-isothiocyanate (FITC) conjugated with G-rich single strand DNA. Based on the principle of chemiluminescence resonance energy transfer (CRET) as shown in Fig. 1(c), FITC excited by dim chemiluminescence generated from the reaction between G-rich single strand DNA and TMPG emitted bright green light (Yamasuji et al., 2011). The results indicate that it is possible to develop highly sensitive biosensors with guanine chemiluminescence detection.

Fig. 1 indicates that TBA, G-rich single strand DNA, can be transformed to G-quadruplex in the presence of thrombin as well as react with TMPG to emit glow chemiluminescence. Using the combination of two apparently distinctive roles of TBA, it is possible to develop G-quadruplex DNA aptasensor with guanine chemiluminescence detection capable of sensing thrombin in a sample. Based on the hypothesis, cost-effective, rapid, and simple G-quadruplex aptasensor with highly sensitive guanine chemiluminescence detection was developed in this study.

2. Experimental

2.1. Chemical and materials

Thrombin aptamer (TBA) and TBA (5′-6-FAM-GGTTGGTGTGGTTGG-3′) conjugated with 6-carboxyfluorescein (6-FAM) were purchased from Alpha DNA (Montreal, Quebec, Canada). Thrombin was purchased from Sigma-Aldrich (100 UN, Milwaukee, WI, USA). 3,4,5-trimethoxylphenylglyoxal hydrate (TMPG, 97%) was purchased from Matrix Scientific (Columbia, SC, USA). $FeCl_2$ (99%), and $FeCl_3$ (99%), Tetra-*n*-propylammonium hydroxide (TPA, 40% w/w aqueous solution) was purchased from Alfa Aesar (Ward Hill, MA, USA). Graphene oxide (GO, 5.5 mg/ml, Flake size: 0.5–5 μm) was purchased from Graphene Supermarket (Calverton, NY, USA). N,N-Dimethylformamide (DMF) and deionized water purchased from EMD (Billerica, MA, USA). Phosphate buffer solution (1.0 M, pH 7.0) was purchased from Teknova (Holliser, CA, USA). 0 calibrator (human serum) was purchased from Monobind, Inc. (Lake Forest, CA, USA).

2.2. Procedure

2.2.1. Interaction between TBA and GO in guanine chemiluminescence

100 nM TBA or TBA conjugated with 6-FAM was prepared in water. GO (110 μg/ml) was prepared in phosphate buffer (5 mM, pH 7). TPA (0.01 M) was prepared in water. TMPG (2 mM) was prepared in DMF. TBA solution (100 μl) was mixed with GO solution (100 μl) in a 1.5-ml microcentrifuge tube. The mixture was incubated for 10 min. After the incubation, the mixture (20 μl) was mixed with TPA (10 μl) in a borosilicate test tube (12 × 75 mm). The test tube was inserted into the sample holder of luminometer with two dispensers (Lumat LB 9507, Berthold Technologies). After touching the start button of the luminometer, the test tube was moved into the detection area. Then, TMPG (200 μl) was injected using the dispenser of luminomer. Finally, light emitted in the test tube was measured for a certain time (e.g., 25, 50, 100 s).

2.2.2. Fabrication of magnetic Fe_3O_4 GO nanoparticles

Magnetic Fe_3O_4 GO nanoparticles were prepared with the modification of method to fabricate magnetic Fe_3O_4 GO/polystyrene nanocomposite (Kassaee et al., 2011). $FeCl_2$ (0.44 mg) and $FeCl_3$ (0.11 mg) were dissolved and mixed in water (5 ml). GO (1.1 mg/ml) was prepared in water. The mixture of $FeCl_2$ and $FeCl_3$ (0.5 ml) was mixed with GO (0.5 ml) in a 1.5 ml-centrifuge tube. The microcentrifuge tube inserted into a Micro Centrifuge Tube Thermomixer (Eppendorf) was shaked for 10 s at 85 °C. Then, ammonium hydroxide (20 μl, 30%) was dispensed into the microcentrifuge. The microcentrifuge tube in the themomixer was shaken at 1400 rpm for 50 min at 85 °C. Magnetic Fe_3O_4 GO nanoparticles formed in the microcentrifuge tube were cooled at room temperature. Magnetic Fe_3O_4 GO nanoparticles in the microcentrifuge tube were washed 3 times in water using a magnetic separator (Bioclone, Inc.).