Biosensors and Bioelectronics 85 (2016) 300–309

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

A versatile nanomachine for the sensitive detection of platelet-derived growth factor-BB utilizing a G-quadruplex-selective iridium(III) complex

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ARTICLE INFO

Article history:
Received 9 February 2016
Received in revised form
13 April 2016
Accepted 2 May 2016
Available online 7 May 2016

Keywords:
G-quadruplex
Iridium(III) complex
Nanomachine
PDGF-BB
Luminescent

ABSTRACT

The novel iridium(III) complex **1** was found to be highly selective for G-quadruplex DNA, and was employed for the development of a versatile nanomachine. In the nanomachine, the single-stranded DNA (ssDNA) oligonucleotide ON1 hybridizes with the hairpin DNA oligonucleotide ON2, leading to the formation of a 5′-recessed double-stranded DNA (dsDNA) structure and the simultaneous release of a G-rich DNA sequence, which folds into a G-quadruplex motif that is recognized by complex **1**. Upon the addition of Exo III, the dsDNA substrate is digested leading to the liberation of ON1 ssDNA, which enters a new cycle of the nanomachine. This nanomachine was successfully used to detect PDGF-BB by combination with a simple pre-procedure module, and exhibited a linear response between luminescence intensity and PDGF-BB concentration in the range of 10 to 300 pM ($R^2 = 0.992$), with a limit of detection for PDGF-BB of 10 pM. This assay was highly selective for PDGF-BB over other proteins, and exhibited potential use in biological sample analysis. Moreover, the versatility of the nanomachine was demonstrated by adapting the nanomachine for the detection of thrombin by simply changing the pre-procedure module.

1. Introduction

Biomarkers are indicators of a biological state or condition, which could not only explore the pathogenesis from the molecular level, but also could accurate and sensitive evaluate early damage at a low leveland can sometimes function as early indicators of pathological processes. (Wu and Qu, 2015) The sensitive and specific detection of biomarkers is therefore an important challenge in the clinical field (Wang et al., 2009, 2015a). Enzyme-linked immunosorbent assays (ELISA) are the most commonly used detection method for biomarkers (Swierczewska et al., 2012). However, these methods tend to require multiple steps and/or sophisticated instrumentation, and the protein antibody is usually unstable and/or tedious to prepare.

Nucleic acid aptamer are single-stranded oligonucleotides obtained from in vitro screening by utilizing the SELEX (systematic evolution of ligands by exponential enrichment) method. The synthesis of nucleic acid aptamers is relatively simple and cost-effective, and their robust stability allows them to withstand up to a wide range of pH values and recover their binding abilities even after many cycles of denaturation-renaturation (Gao et al., 2015; Kim et al., 2016). These properties have rendered aptamers as important versatile signal transducing components in DNA-based detection platforms.

However, it is difficult to construct a highly sensitive aptamer-based detection platform through a conventional signal transducing method because of the relatively low binding affinity possessed by certain aptamers towards their target molecules. Hence, it is necessary to design a signal amplification strategy when using relatively weaker aptamers. During the past years, various DNA-based amplification strategies have been developed for biomarker determination, including “the gold standard” polymerase chain reaction (PCR), (Becker-André and Hahlbrock, 1989; Tadokoro et al., 1991) exonuclease III (Exo III)-assisted target recycling amplification (ERA) (Bi et al., 2014; Fan et al., 2012; Hao et al., 2014;

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http://dx.doi.org/10.1016/j.bios.2016.05.026
0956-5663/

Zhao et al., 2012), rolling circle amplification (RCA) (He et al., 2014; Tang et al., 2013; Zhou et al., 2007), strand-displacement amplification (SDA) (Chen et al., 2015; Du et al., 2016; Huang et al., 2015), and hybridization chain reaction (HCR) (Ge et al., 2014; Yang et al., 2012). However, most of them utilized expensive labeled DNA oligonucleotides, while others employ several kinds of enzymes, thus requiring exact environmental conditions and extensive optimization. Besides amplification techniques, non-amplification techniques such as surface-enhanced resonance Raman scattering (SERRS) are also known (Bonham et al., 2007). Non-amplification techniques tend to be less complicated, but may exhibit lower sensitivity, amplification techniques are usually more sensitive, but rather complicated.

DNA machines are biomolecular assemblies which possess the ability to automatically produce a signal fueled by target molecules (Li et al., 2013a; Nakatsuka et al., 2015; Xu et al., 2015a). DNA machines with different functions have been designed, such as switches (Johann et al., 2009), walkers (Kyle et al., 2010; Omabegho et al., 2009), gears (Tian and Mao, 2004) and sensors (Li et al., 2015; Xu et al., 2016, 2015b; Ye et al., 2012; Zhang et al., 2014), and some of these have been coupled to colorimetric, fluorescent, chemiluminescence, surface-enhanced Raman scattering (SERS) strategy, or other outputs. DNA machines have found considerable applications in constructing biosensors, providing a new opportunity for the sensitive and precise detection of target molecules.

Meanwhile, the guanine (G)-quadruplex structure is a type of non-canonical DNA secondary structure consisting of planar stacks of four guanine residues stabilized by Hoogsteen hydrogen bonding. It has been widely used as a signal transducer for the development of label-free sensing platforms (Collie and Parkinson, 2011; Tran et al., 2011). Transition metal complexes have attracted great attention as probes for biomolecule detection, due to their simple synthetic protocols, tunable excitation and emission maxima over the visible region, long lifetimes and large Stokes shifts (Chen et al., 2014; Li et al., 2011; Lim et al., 2009; Liu et al., 2011; Yu et al., 2008). In particular, iridium(III) octahedral complexes have been recently studied as G-quadruplex-selective probes for the development of luminescent sensing platforms for a variety of targets (Lu et al., 2014; Ma et al., 2013; Lin et al., 2015; Wang, et al., 2016). Recently, our group has developed a variety of iridium(III) complexes as G-quadruplex-selective probes for the construction of a range of simple, rapid, sensitive and cost-effective label-free luminescent detection platforms (Leung et al., 2013; Lu et al., 2015; Ma et al., 2013, 2014).

Encouraged by these strategies, we report herein the development of a novel iridium(III) complex **1** as a highly selective G-quadruplex probe, which was utilized for the establishment of a label-free nanomachine for the sensitive determination of platelet-derived growth factor BB (PDGF-BB). Human PDGF-BB is an important cytokine that is involved in growth and progression, tumor transformation, and is thus a protein marker for cancer diagnosis. Hence, the sensitive detection of PDGF-BB is of great importance (Chang et al., 2013; Fang et al., 2015, 2001; Hart et al., 1990; Huang et al., 2016; Kim et al., 2009; Li et al., 2013b). Our detection strategy employs an Exo III-assisted nanomachine that can be readily modified in order to achieve the label-free determination of different biomolecules. To our knowledge, this is the first versatile nanoDNA machines utilizing iridium(III) complex for target.

2. Materials and methods

2.1. Materials and apparatus

PDGF-BB was purchased from ProSpec-Tany Technogene Ltd. (Israel). β-amyloid, thrombin, lysozyme, trypsin and hemoglobin were purchased from Sigma Aldrich (St. Louis, MO). HIF-1α is purchased from Sino Biological Inc. (Beijing, China). PDGF-BB was dissolved in 4 mM HCl and then diluted in Tris buffer before use. The stock solution of PDGF-BB (Molecular Weight: 32,021 Da) is prepared by dissolving 10 μg of PDGF-BB in 100 μL 4 mM HCl, then the solution is diluted with 524 μL Tris–HCl buffer (20 mM Tris, 100 mM KCl, pH 7.4), so the calculated concentration should be 0.5 μM. Iridium chloride hydrate (IrCl3.xH2O) was purchased from Precious Metals Online (Australia). All oligonucleotides were synthesized by Techdragon Inc. (Hong Kong, China). Exo III and Exo I were purchased from New England Biolabs Inc. (Beverly, MA, USA). Other reagents, unless specified, were also purchased from Sigma Aldrich (St. Louis, MO).

All of the emission spectra for complex **1** were performed on a PTI TimeMaster C720 Spectrometer (Nitrogen laser: pulse output 337 nm) fitted with a 380 nm filter. Error limits were estimated: λ (± 1 nm); τ (± 10%); ϕ (± 10%). Emission and excitation slits were sent as 2.5 nm, and all of the detections were carried out at room temperature (25 °C).

Circular dichroism (CD) spectra were recorded on a JASCO-815 spectropolarimeter using 1 cm path length quartz cuvettes. Spectra were collected between 220 nm and 335 nm, using 2 cm bandwidth, 100 nm min^{-1} scan speed and five scans. The data were baseline corrected using CD spectra of buffer alone.

FRET-melting experiment was carried out by using a Mx3005 P QPCR system (Agilent technologies) in a 96-well format (quartz tungsten halogen lamp excitation source, PMT detector). The data was processed by Mx3005 P QPCR software (version 4.10).

2.2. Methods

The procedures for detection of PDGF-BB by modified nanomachine were presented as follows. The ssDNA ON3 (50 μM) and ON4 containing the PDGF-BB aptamer sequence (50 μM) were mixed and heated to 95 °C for 10 min, and allowed to cool slowly to room temperature over the period of 1 h to form the ON3/ON4 dsDNA structure. The annealed DNA was stored at −20 °C before use. For PDGF-BB detection, different concentrations of PDGF-BB were incubated with 0.4 μM of ON3-ON4 dsDNA in 50 μL Tris–HCl binding buffer (20 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1 mM $MgCl_2$) at 37 °C for 0.5 h, leading to the formation of the PDGF-BB/ON4 complex and the release of the ssDNA ON3. Then, 0.5 μM of hairpin DNA ON2 was injected into the reaction system, and the system was incubated at 37 °C for 0.5 h, leading to the formation of ON3/ON2 dsDNA and the release of the G-rich DNA sequence in ON2. After that, 5.5 μL 10 × NEBuffer 1 (100 mM Bis-Tris-Propane-HCl, 100 mM $MgCl_2$, 10 mM DTT, pH 7.0) and 0.4 μL Exo III (40 units) were added into the reaction mixture to perform the Exo III-aided amplification reaction. After 45 min at 37 °C, Exo III was then inactivated by heating the system to 90 °C for 10 min, and subsequently, 75 mM of potassium ions was added to induce the formation of the released G-rich sequences into a G-quadruplex structure. Finally, the reaction solution was diluted to 500 μL with Tris–HCl buffer (10 mM Tris–HCl, pH 7.4), and 0.75 μM complex **1** was added to the mixture. Emission spectra were recorded in the 480–760 nm range using an excitation wavelength of 310 nm.

For the detection of PDGF-BB in diluted serum, 5 μL of human serum was added into 45 μL binding buffer (20 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1 mM $MgCl_2$). Then, different concentrations of PDGF-BB were incubated with 0.4 μM of ON3-ON4 dsDNA in the serum containing system. The detection procedures were performed as above.

For the detection of PDGF-BB using the organic dye ThT, the procedures were the same as described above, except that 2 μM of ThT was used to replace 0.75 μM of complex **1**. The emission spectra were recorded in the 450 − 620 nm range using an excitation wavelength of 435 nm.