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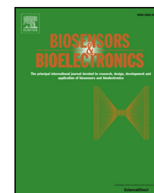

Target-triggered three-way junction in conjugation with catalytic concatemers-functionalized nanocomposites provides a highly sensitive colorimetric method for miR-21 detection

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ABSTRACT

With the great advances in DNA nanotechnology, scientists have shown interest in developing dynamic nanostructures for theranostic applications, analyte sensing and cargo delivery. Here, we present a specific enzyme-free ultrasensitive platform based on a multilayer coupled signal amplification strategy to quantify miR-21 molecule. The biosensor was integrated based on three signal amplification gadgets, namely a translator-mediated catalytic hairpin assembly (CHA), a multilayer DNA concatemer on the surface of gold decorated magnetic nanoparticle (GMNP), and a DNAzyme-mediated catalytic signal amplification. MiR-21 mediates the release of a DNA translator from an immobilized duplex to engage in a CHA reaction using three hairpins, including a GMNP-conjugated hairpin 1 (H1), biotin-labeled hairpin 2 (H2) and a GMNP-conjugated hairpin 3 (H3) to form a three-way junction (3WJ). Meanwhile, a plenty of initiator strand 0 (S0) on GMNPs – each of which has been bifunctionalized with S0/H1 or S0/H3 – drive several multilayer peroxidase-mimicking DNAzyme concatemers in the presence of two accessory oligonucleotides; strand 1 (S1) and strand 2 (S2). Since a G-rich sequence was attached at the 5′-end of S1 strand, in the presence of hemin cofactor, an active G-quadruplex DNAzyme with peroxidase activity was formed. The concatemers on the surface of GMNPs can convert a colorless substrate to a green product. The biosensor can detect as low as 1 aM of miR-21 and provide an excellent capability to discriminate single-base mismatches. The required time for the formulation of the assay reagents is about three days and the reaction time for the detection of miR-21 takes place in less than four hours.

1. Introduction

Dynamic nucleic acid nanotechnology is built on kinetically controlled DNA strand-displacement reactions, mediated by either enzyme/DNAzyme (Cai et al., 2017; Wickham et al., 2011), chemical-trigger interactions (Wang et al., 2014) or autonomous self-assembly processes (Yin et al., 2008). As a result of their implications for various biomaterial systems, such as information processing (Ravan et al., 2017; Seelig et al., 2006), analyte sensing/signal amplifying (Fozooni et al., 2017; Norouzi et al., 2018; Zhang et al., 2016; Choi et al., 2011; Huang et al., 2014), nanoactuators (Simmel and Yurke, 2001) and cargo delivery (Song et al., 2013), dynamic nanodevices have received considerable attention in recent years. Signal amplification through autonomous DNA self-assembly provides a simple and powerful framework for analyte detection (Zheng et al., 2018) due to the feasibility in design, execution and tuning. Among the most widely used dynamical signal amplification strategies are entropy-driven catalysis (EDC) (D.Y. Zhang et al., 2007), hybridization chain reaction (HCR) (Dirks and Pierce, 2004; Choi et al., 2014) and catalytic hairpin assembly (CHA) (Yin et al., 2008), which tend to exploit kinetically metastable DNA monomers like multi-stranded complexes and hairpins, in order to produce a stable DNA nanostructure. However, coupling the formation of these nanostructures with an appropriate signal transducer has led to the generation of exponential signal amplifications in response to target analytes.

Pioneered by Pierce's group, the autocatalytic target-triggered self-assembly of hairpin monomers through the HCR and CHA technologies has been broadly used within the context of dynamic DNA nanotechnology for signal amplifications. Several research groups have integrated these principal components with different nanomaterials

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(Huang et al., 2014; Zhao et al., 2017), polymer (Li et al., 2012;), functional nucleic acid (Wang et al., 2011) and enzyme-assisted platforms (Song et al., 2015; Tao et al., 2015) for the analysis of various biomolecules. For instance, Huang et al. (2011), designed a DNA-responsive fluorescent switch based on pyrene excimer formation upon HCR completion. Zhang et al. (2012) developed an improved immuno-HCR assay using bi-functional gold nanoparticles (GNP) and magnetic microparticles in a sandwich format for ultrasensitive detection of human IgG. In a similar strategy, Hau et al. (2015), designed an HCR-based DNAzyme concatemer on GNPs for colorimetric detection of carcinoembryonic antigen. To improve the analytical performance of enzyme-free signal amplification strategies, Wang et al., used CHA and HCR in different studies to induce aggregation of modified GNPs for detection of nucleic acid and adenosine targets (Quan et al., 2015a, 2015b). This group also fabricated hairpin-locked-DNAzyme modified GNPs for detecting miRNA in living cells (Yang et al., 2017). Miao et al. also developed target-stimulated concatemer formation on an electrode-modified DNA tetrahedral nanostructure to detect miRNA molecules (Miao et al., 2015). In another strategy, Ravan designed a modified HCR to construct a multilayer DNAzyme concatemer on GNP for the detection of 16 s rRNA molecules in E. coli bacteria (Ravan, 2016a).

To achieve a powerful biosensing technology with improved sensitivity, specificity and wider dynamic range, a multilayer-integrated signal amplification process needs to be examined. With this in mind, a novel combination of signal amplifiers cached our attention to design an upgrade colorimetric biosensor for the detection of a miRNA with enhanced analytical performance. In this study, we exploited multiple signal amplification strategies to detect miR-21 (Tian et al., 2018) – a short 22-nucleotide RNA molecule which is overexpressed in several human cancers (Wang, 2009; Shah and Calin, 2014) – through the self-assembly of GMNP-labeled oligonucleotides by a CHA reaction. The GMNP-labeled oligonucleotides provide a foundation for the construction of a multilayer G-quadruplex DNAzyme, which in the presence of the hemin molecule produces a colored product. To build this system, two groups of GMNPs were prepared, which were bi-functionalized with hairpin H1/initiator S0 and hairpin H3/S0 (Scheme 1). Hairpin H1 and H3 on different GMNPs formed a catalytic three-way junction self-assembly by recruiting hairpin H2 as the bridge of two GMNPs in the presence of a universal translator as the trigger of the self-assembly process. The single stranded S0 on the GNMPs served as the initiator of the formation of DNA concatemers. The concatemers bore many G-quadraplex domains which produced active DNAzymes with peroxidase activity in the presence of hemin cofactor. In this way, an exponential signal amplification was achieved through (i) the formation of three-way junctions (3WJs), (ii) the formation of multiple DNA concatemers on GMNPs with multilayer G-quadraplex DNAzymes and (iii) the catalytic activity of peroxidase-mimicking DNAzymes to produce a plenty of color products. Through this strategy, attomolar concentrations of the miR-21 can be measured by spectrophotometry.

2. Experimental section

2.1. Materials and reagents

HPLC or PAGE-purified oligonucleotides were synthesized by Bioneer Co. (South Korea), dissolved in deionized water and stored at −20 °C until use. The sequence of oligonucleotides is represented in Table S1. 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Tris(2-carboxyethyl) phosphine hydrochloride (TCEP), Hemin, Fe_2O_3, tetrachloroauric (III) acid trihydrate, bovine serum albumin (BSA), streptavidin, dimethyl sulfoxide (DMSO), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and trisodium citrate were purchased from Sigma-Aldrich Co. (USA). Hemin stock solution (0.1 mM) was prepared in DMSO and stored in a dark bottle at −20 °C until required. All aqueous solutions were prepared with deionized water with electrical resistivity of 18.25 (MΩ cm^{-1}). Formation of DNA nanostructure was checked by electrophoresis in a 2.5% agarose gel using 1 × tris-acetate-EDTA buffer at a constant voltage of 80 V for 120 min. The gel was stained by ethidium bromide and visualized using UV-Doc HD 6 light box.

2.2. Preparation and characterization of gold decorated Fe_2O_3 nanocomposites

Gold decorated Fe_2O_3 nanoparticles were synthesized according to Murph's method by using citrate reduction of chloroauric acid (Larsen et al., 2016; Murph et al., 2016). In brief, 100 µL of 25 mM Fe_2O_3 colloid was injected into 10 mL of deionized water under gentle stirring. The mixture was heated for approximately 5 min and 1 mL of 1% citrate solution was added. To the boiling solution, 250 µL of 0.01 M chloroauric acid was added and after 10 min the solution color changed from burnt orange to deep red which proved the formation of Au nanoparticles. The solution was allowed to cool to room temperature and the GMNPs were collected using an external magnet. The separated GMNPs were suspended in deionized water for future use.

2.3. Bi-functionalization of GMNPs with S0/H1 or S0/H3 oligonucleotides

At first, the alkanethiol-caped S0, H1 and H3 oligonucleotides (100 µM) were added to the separate tubes, heated to 95 °C and slowly cooled to room temperature. Then the caped oligonucleotides were converted to active alkanethiol derivatives by adding a solution containing 10 mM TCEP, 10 mM Tris-HCl, 1 mM EDTA and 0.1 M NaCl (pH 7.4). The activated molecules were incubated with GMNPs according to a previously published protocol (J. Zhang et al., 2007). Briefly, 1 mL of GMNPs was incubated with S0/H1 or S0/H3 at different ratios (90:10, 80:20, 70:30, 60:40 and 50:50 with a final concentration of 3 µM) for 16 h at room temperature. To get the maximum loading of the oligonucleotides on GMNPs, salting buffer (10 mM phosphate buffer containing 2.0 M NaCl, pH 7.0) was added dropwise to reach the final salt concentration of 0.1 M NaCl. The mixture was incubated at room temperature for 40 h. Then, the bi-functionalized GMNPs were separated by an external magnet and resuspended in 100 µL of the hybridization buffer (10 mM phosphate buffer supplemented with 200 mM NaCl, 30 mM KCl, and 10 mM $MgCl_2$).

2.4. Colorimetric detection of miR-21

MiRNA-directed signal amplification is limited by its natural features such as vulnerability to degradation and small size (Liao et al., 2015). To overcome these issues, a DNA duplex with translation activity was used (Ravan, 2015). At first, the translator was confined at the surface of microtiter plate wells. For this purpose, each well was incubated with 100 µL of 10 µg/mL streptavidin in RNase-free phosphate buffered saline (PBS) at 4 °C overnight. The wells were then blocked with 250 µL of 1% (w/v) BSA in PBS and then washed out (Ravan and Yazdanparast, 2013, 2012a). To the wells, 100 µL of a biotinylated universal translator – which was pre-formed by heating a mixture of capture and translator oligonucleotides (each 1 µM) to 95 °C and slowly cooled to room temperature – was added and incubated for 30 min.

Different concentrations of miR-21 (80 µL) were added to the wells and incubated for 30 min at room temperature. Based on toehold-mediated DNA strand displacement reaction (TMSD), the translator oligonucleotide is displaced by miR-21 and released in the solution. To the supernatant (70 µL) that contains the translator oligonucleotide, 10 µL biotinylated H2 (1.5 µM), 10 µL H1/S0 bi-functionalized GMNP and 10 µL H3/S0 bi-functionalized GMNP were added and incubated at 25 °C. After 2 h, the GMNPs were settled at the bottom of the tube by a magnet and the solution containing the H2 monomers, which were not used in the 3WJ formation, was discarded. Subsequently, the separated GMNPs were re-suspended in 100 µL hybridization buffer and added to streptavidin-coated wells. After 30 min incubation, the supernatant was

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