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A simple and rapid detection assay for peptides based on the specific recognition of aptamer and signal amplification of hybridization chain reaction

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ABSTRACT

A simple and rapid assay for the detection of peptides is designed based on the specific recognition of aptamer, the quenching effect of graphene oxide (GO) and the efficient signal amplification of hybrid chain reaction (HCR). In this assay, the hairpin structure of aptamer is opened after binding with targets, and the initiation sequence could be exposed to hairpin probe 1 (H1) to open its hairpin structure. Then the opened H1 will open the hairpin structure of hairpin probe 2 (H2), and in turn, the opened initiation sequence of H2 continues to open H1. As a result, the specific recognition of target and fluorescent signals are accumulated through the process in short 1 h. Attentively, the aptamer can not only identify target peptides, but also initiate the HCR between H1 and H2. More importantly, the HCR is initiated only after the target recognition of aptamer. After HCR, the excess hairpin probes will be anchored on the GO surface, and the background is greatly reduced due to the quenching effect of GO. By using Mucin-1 (MUC1) as a model peptide, the assay has a wide linear range as two orders of magnitude and the detection range is from 0.01 to 5 nM with low detection limit of 3.33 pM. Therefore, the simple and rapid detection of the target can be realized, and the novel assay has great potential in detecting various peptides and even cancer cells.

1. Introduction

Sensitive quantification of peptides, which are related to cancers especially, has important significance in early warning and diagnosis of disease (Jian et al., 2010; Knutson et al., 2001). In the past years of research, various detection methods have been developed, including enzyme-linked immunosorbent assay (Aydin, 2015), label-free SPR detection (Soler et al., 2016), LC-MS (Sealey-Voyksner et al., 2016), far-ultraviolet absorbance (Uchiho et al., 2015), electrochemical analysis (Enache and Oliveira-Brett, 2013). Though these multifarious methods have high sensitivity and specificity, they are fussy and time-consuming, requiring a series of immobilization, separation, and washing steps, and needing expensive enzyme. In these regards, aptamer recognition and enzyme-free amplification methods are rather simple and low in cost, which are more attractive.

Aptamers are one kind of short oligonucleotide sequences (small DNA or RNA) that could be in vitro screened and synthesized with systematic evolution of ligands (Green et al., 1990; Tuerk and Gold, 1990). With superior properties such as high affinity and strong specific binding with corresponding ligands, it provides a new fast and efficient recognition research platform for chemical biology and biomedical community, and in many ways shows a good application prospect. As of today, aptamers have been discovered specific binding affinity toward a variety of targets ranging from peptides (Cho et al., 2015; Kirby et al., 2004), small molecules such as organic dyes (Ellington and Szostak, 1990; Iliuk et al., 2011; Zhu et al., 2015) and even some cells (Bayrac et al., 2011; Dimitri et al., 2010; Keefe et al., 2010; Sefah et al., 2009). To the subsequent later, the current years have witnessed quantity of progresses in the application of aptamers to develop aptasensors (Feng, 2014; Sharma, 2015; Van den Kieboom et al., 2015 ; Zhou, 2010).

The mechanism of the signal enhancement and background reduction is vital for targets to make better understanding of their analytical information. On the one hand, to open up and get more fluorescent signals, amplification strategy is needed. HCR has shown great potential in nucleic acid detection as an attractive amplification method (Choi et al., 2014; Elenis et al., 2007; Zhang

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et al., 2012), which makes it that the amplification does not require any enzymes. On the other hand, graphene oxide (GO) is well-known for its extraordinary electronic, optical, and thermal properties as well as large surface area, good water dispersibility and biocompatibility (Borini et al., 2013; Lim et al., 2015; Liu et al., 2011; Ray et al., 2015; Saxena et al., 2010; Suk et al., 2010; Zhang et al., 2011a). Compared with other nanomaterials, a superior properties of GO, high quenching efficiency, makes it possible as quenching reagent because of its electrostatic force and π-π interaction with peptides and oligonucleotides as well as the quenching effect on absorbed fluorescent dyes (Zhang et al., 2011b; Bianying et al., 2013; Huang et al., 2014; Yang et al., 2012).

Based on the above considerations, we designed a simple and rapid assay for the detection of peptides. In this assay, the hairpin structure of aptamer is opened only after binding with targets, and the initiation sequence could be exposed to H1 to open its hairpin structure. Then the opened H1 will open the hairpin structure of H2, and then opened initiating sequence of H2 continues to open the hairpin structure of H1. As a result, the specific recognition of target and fluorescent signals are accumulated through the process in short 1 h. More importantly, the aptamer can not only identify target peptides, but also initiate the HCR, which is initiated only after the target peptide recognition by aptamer. And the background is greatly reduced due to the quenching effect of GO as its electrostatic force and π-π interaction with peptides. Meanwhile, this simple and rapid assay can be completed in very short time without expensive enzymes.

2. Experimental section

2.1. Materials and apparatus

All of the reagents used in this work were of analytical grade and used as purchased without further purification. Graphite oxide was purchased from XFNANO, INC (Shanghai, China). All of the oligonucleotides and the MUC1 peptide used in experiments were synthesized by Sangon Biotechnology Co. Ltd. (Shanghai, China). All fluorescence measurements were performed on FL920 Fluorescence Spectrometer.

The sequences used in the HCR system were listed as below (from 5′ to 3′, it is noted that sticky ends are underlined and loops are italicized, and the initiating sequence of aptamer is bold).

H1: AGTAGGTTGTATAGTT*CAAAGT*AACTATACAACCTACTACCTCA-FAM

H2: FAM-ACTTTGAACTATACAACCTACT*TGAGGT*AGTAGGTTGTATAGTT

Aptamer:
TGAGGTAGTAGGTTGTATAGTTGCAG*TTG*ATCC*TTT*GGAT*ACC*C TGGAACTATACAACCTACTACCTCA.

2.2. Reaction procedures of the assay

Firstly, the FAM-labeled hairpin DNA probes (H1 and H2) and aptamer sequence were heated at 95 °C for 2 min, respectively. And they were treated to cool to room temperature for 1 h before use. The following procedure was processed in the final volume of 200 μL reaction solution. Different concentrations of MUC1 peptides were mixed with 50 nM H1 and 50 nM H2 in SPSC buffer (0.75 M NaCl, 50 mM Na_2HPO_4, pH 7.4) for 1 h at room temperature to proceed the HCR. Subsequently, GO was added into the HCR system and the final mixture was further incubated for 30 min.

2.3. Detection procedures of the assay

Finally, fluorescence signals were recorded with experiment parameters as follows: the excitation wavelength was 480 nm and the emission wavelengths were in the range from 500 to 650 nm.

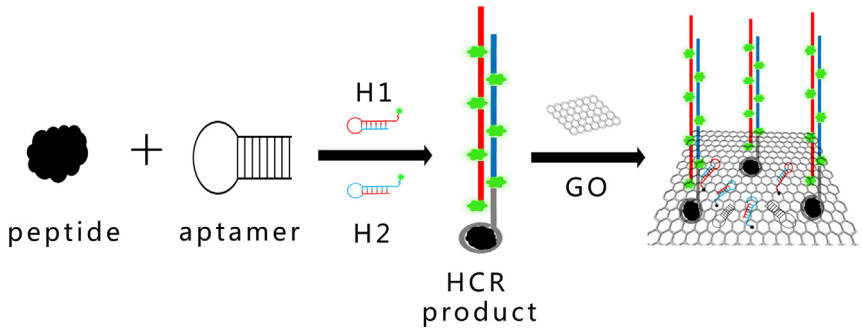
3. Results and discussion

3.1. Schematic diagram of experimental principle

The new proposed method for the detection of peptides is illustrated in Scheme 1, and MUC1 is used as model peptide. With each bearing a fluorophore at the sticky terminal, a pair of hairpin DNA probes (H1 and H2) are specifically designed for the HCR. The aptamer, which could specifically bind to MUC1, owns a sequence that could initiate the HCR between H1 and H2. In the absence of MUC1, aptamer, H1 and H2 can be coexisted stably in the solution. When MUC1 is present, it binds with aptamer specifically, whose structure could be changed (Ferreira et al., 2006). As a response, the initiated sequence of opened aptamer hybridizes with the sticky end of H1 and opens the hairpin through an unbiased strand-displacement interaction. The newly released sticky sequence of H1 will further hybridize with the sticky end of H2 to open its hairpin and expose a new sticky end on H2. The sequence of this sticky end is identical to that opened sequence of aptamer. In this manner, each MUC1 molecule can specifically open one initiated sequence of aptamer, which could trigger a chain reaction of hybridization events between H1and H2. With the presence of GO, the excess H1 and H2 would be closely adsorbed onto GO surface via π−π stacking (Dubuisson et al., 2011). As a result, a quantity of accumulated fluorescence signals is yielded.

3.2. Experimental proof of the proposed assay

As shown in Fig. 1A, it can be seen from the green line that



Scheme 1. Schematic illustration of the proposed assay for peptide detection, using MUC1 as a model peptide.