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Label-free and enzyme-free fluorescent isocarbophos aptasensor based on MWCNTs and G-quadruplex

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ABSTRACT

A novel label-free and enzyme-free detection strategy has been developed for the fluorescent detection of isocarbophos (ICP) using multi-walled carbon nanotubes (MWCNTs) and G-quadruplex as the signal transducers. In this work, the split ICP aptamer were attached to a G-quadruplex motif at their respective terminals. In the presence of ICP, the split aptamers could undergo conformational change into a sandwiched-like ternary complex, which prevent them from adsorbing to the MWCNTs due to the increased steric hindrance. As a result, the fluorescence signal of the G-quadruplex probes N-methyl mesoporphyrin IX (NMM) was enhanced significantly. In the absence of ICP, the split aptamer only existed in the form of single-stranded DNA, which could be easily adsorbed by MWCNTs and resulted in a quenched fluorescence signal of NMM. The proposed strategy could selectively and sensitively detect ICP with a detection limit of 10 nM. Furthermore, we have also demonstrated the capability of this strategy in the detection of ICP in real samples from vegetable extract, indicating the potential application of this strategy in food safety issues.

1. Introduction

Organophosphorus pesticides (OPPs) have been widely employed in agriculture and households attributed to their ability to protect plants from pests and diseases. However, OPPs is known for its acute toxicity and environmental hazard over the past few decades. For example, OPPs could be continually assimilated by plants and accumulated in animals and humans, which could threaten human health even at a very low concentration [1–4]. Therefore, many regulations have been implemented to restrict the level of OPPs residue worldwide [5]. As one of the widely used OPPs, isocarbophos (ICP) has been perceived its residues in various foods, such as rice, vegetable and fruit. Importantly, it has been reported that long-term exposure of ICP can cause vascular cognitive impairment in rats [6,7]. Considering its high toxicity, it is pivotal to establish a simple, sensitive, rapid and cost-effective sensing platform for the assay of ICP to relieve the concerns in food safety and environmental protection.

To date, numerous methods have been reported for the detection of OPPs, including capillary electrophoresis (CE) [8], chromatography [9,10], colorimetric spectroscopy [11–13], electrochemical technique [14,15] and fluorescent sensors [16,17]. Among these strategies, fluorescence-based methods have attracted great interest due to their high sensitivity, simplicity, cost-effectiveness and easy operation [18]. For example, the combination of chemosensors with enzymes is the commonly used strategies for the detection of OPP. However, these methods were usually restricted in real application, since enzymes is always susceptible to the complicate detection conditions, such as temperature, solution pH and solvent species, and the immobilizations of enzymes on the surface of materials or electrodes are also in certain difficulties [19–21]. Therefore, many efforts have been devoted to explore an enzyme-free strategy for the detection of OPP. Meanwhile, carbon nanomaterial is appeared as a promising tool in the development of enzyme-free biosensors [22–24]. Single-walled carbon nanotubes (SWCNTs), MWCNTs and various modified grapheme oxide (GO), have also been explored as a "nanoquencher" of fluorophores [25–27]. Equipped with aptamer techniques, these carbon nanomaterials can be employed to construct versatile fluorescent nanosensors for various targets detection [28–31]. When comparing with SWCNs, MWCNTs also have the property of one-dimensional (1D) structure, but with a larger diameter. It is reported that the wrapping and adsorption of ssDNA around SWCNTs is slower than that on MWCNTs [32]. Hence, we inferred that MWCNTs employed in this assay could efficiently and quickly quench the fluorescence of the G-quadruplex probe NMM.

Aptamers are artificial and functional DNA/RNA oligonucleotides that screened by exponential enrichment techniques [33]. In the field of biosensors, aptamers offered many advantages, such as strong affinity, simple synthesis, low cost and high stability under various conditions [34–37]. A dominant strategy for protein detection is the sandwiched-

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type biosensors due to their high specificity and sensitivity endowed by the dual recognition mechanism. However, this strategy is not suitable to assay small molecules due to the existing steric hindrance, which could hinder the simultaneous binding of small molecules to the two aptamers. To overcome this problem, stojanovic and co-workers proposed the concept of split nucleic acid aptamer in 2000, in which aptamer could be cut into two parts without affecting their ability to transform into a three-dimensional target/aptamer complex upon the addition of target [38]. This seminal strategy has been widely employed to detect various targets, especially small molecular targets with long chain of oligonucleotide aptamers [39–41].

Moreover, G-quadruplex-based detection strategy has been widely used in a variety of detection platforms due to their label-free properties [42–46]. Furthermore, N-methyl mesoporphyrin IX (NMM) is one of the widely used fluorophore, which only produces strong emission at 611 nm upon binding with G-quadruplex. As a result, NMM could afford detection with high signal/noise ratio, which is critical in the construction of a highly sensitive platform.

Inspired by these concepts, we aim to develop a novel label-free and enzyme-free fluorescent ICP assay based on MWCNTs and G-quadruplex. ICP is a small molecule, but its aptamer is a rather long DNA chain (5′-$A_2GCT_6GACTGACTGCAGCGAT_2CT_2GATCGC_2ACG_2TCTGGA_5$ GAG-3′), containing 54 bases [47]. In this work, split aptamer of ICP was employed to avoid false-positive or nonspecific interactions between ICP and its aptamer, as well as reduce the synthetic cost of long chain DNA. To construct the G-quadruplex based label-free detection platform, G-rich DNA sequences were attached to the two terminals of the ICP aptamers. Initially, the two split ICP aptamers oligonucleotide (Apt-1 and Apt-2), which include G-quadruplexes forming sequences exist in the form of free single-stranded DNA, which could be easily adsorbed by MWCNTs and resulted in a quenched fluorescence signal of NMM. However, in the presence of ICP, the two G-quadruplex tailed split aptamers could undergo conformational change into a sandwiched-like complex, which prevent them from adsorbing to the MWCNTs due to the increased steric hindrance. As a result, the fluorescence signal of the G-quadruplex probe NMM was enhanced significantly. Based on this strategy, a simple, selective, sensitive, label-free, and enzyme-free detection assay for ICP was developed. Furthermore, we have also demonstrated the capability of this strategy in the detection of ICP in real samples from vegetable extract, indicating the potential application of this strategy in food safety issues. To the best of our knowledge, label-free and enzyme-free detection of ICP based on MWCNTs and G-quadruplex has not yet been reported in literatures

2. Methods

2.1. Chemicals and materials

Isocarbophos (98.6%, purity) was purchased from TCI (Shanghai). Other pesticides, including methidathion, imidacloprid, chlorpyrifos, phoxim, acetamiprid, thiram, diethofencarb, atrazine, metalaxyl and ziram, were bought from Aladdin (Shanghai, China). MWCNTs was obtained from Shenzhen Nanotech Port Co. Ltd. NMM was bought from J&K Scientific Ltd. (Beijing, China), and was dissolved in ultrapure water with the concentration of 250 μM. Other chemicals were purchased from BODI (Tianjin Chemical Reagent Co. Ltd). All oligonucleotides (ULTRAPAGE grade) were synthesized and purified by Sangon Biotech Co., Ltd (Shanghai, China), which were listed in Table 1.

For the processing of DNA, the lyophilized powders were dissolved in 10 mM Phosphate (PBS) buffer and stored at − 20 °C until use. PBS (0.01 M) with pH = 7.4 were prepared through diluting stock standard solutions (containing 0.2 M NaH_2PO_4, 0.2 M Na_2HPO_4, 3.0 M NaCl, 0.1 M $MgCl_2$, 0.5 M KCl;). All chemicals and solvents were at least of analytical grade and used as received without further purification. Ultrapure water (resistivity > 18.2 MΩ/cm at 25 °C) obtained from a Milli-Q water purification system (Millipore Corp., Bedford, MA, USA) was used throughout the experiments.

Table 1
DNA sequences used in this work.

DNA	Sequence
Apt-1	5′- **$GTG_3TAG_3CG_3T_2G_2$**$T_2A_2GCT_6GACTGACTGCAGCG$ −3′
Apt-2	5′- ***$AT_2CT_2GATCGC_2ACG_2TCTG_2A_5GAG$***$A_2$**$GTG_3TAG_3CG_3T_2G_2$**–3′

The bold bases are G-rich DNA sequences of PS2.M, the italic underlined bases are split aptamer sequences of ICP.

2.2. Pre-treatment of MWCNTs

The procedure of purification and oxidation of MWCNTs was carried out according to the method described by He and Bayachou [48]. 150 mL HNO_3 (2.0 M), containing 200 mg MWCNTs, was refluxed for 24 h. Then, the mixture was centrifuged at 14,000 rpm for 30 min, and the supernatant was removed. The precipitates were further oxidized by 40 mL of HNO_3 (68%)/H_2SO_4 (98%) with the proportion of 1: 3 in an ultrasonic bath for 4 h. Then, the reactants were diluted 10 times with water and kept for 12 h at room temperature. After removal of the upper clear solution, the remaining suspension was filtered by a 0.45 μm filtration membrane and then washed with water until the filtrate was neutral. The resulted precipitates were dried under vacuum for about 6 h. The obtained MWCNTs with carboxyl groups were re-dispersed in water to give a final concentration of 5 mg/mL.

2.3. Fluorescence assay of ICP detection

Firstly, Apt-1 and Apt-2 were heated individually at 95 °C for 5 min and then allowed to cool to room temperature for 1 h before use. Then, different concentrations of ICP and 100 nM of Apt-1/Apt-2 were incubated in the binding buffer (50 mM NaCl, 10 mM KCl, 10 mM $MgCl_2$, and 50 mM Tris/HCl, pH = 8.0) for 30 min at room temperature to proceed the binding event between ICP with Apt-1/Apt-2. Secondly, concentrated MWCNTs were added into the system with the final concentration of 90 μg/mL, and the mixture was further incubated at room temperature for 30 min. Finally, 8 μL of NMM (250 μM) was added into the system. The final volume of the reaction solution reached 500 μL by dropping into certain volume of 50 mM Tris/HCl buffer. It should be noted that all of the mentioned concentrations are based the final volume 500 μL. Afterwards, the fluorescence spectra were recorded on a fluorescence spectrometer.

2.4. Real sample preparation

Fresh Chinese cabbages and apples were used as the real samples matrix to investigate the practical potential of this aptasensor, which were purchased from the local market (Qingdao, China). Firstly, 10 g edible part of cabbage was homogenized in solution containing 5 mL water and 20 mL acetonitrile. After 10 min, the mixture was brought into an ultrasonic bath for 30 min and then filtrated. The filtrate was concentrated by rotary evaporation at 70 °C. Lastly, the obtained solution was re-dissolved in the detection buffer (50 mM NaCl, 10 mM KCl,10 mM $MgCl_2$, and 50 mM Tris/HCl, pH = 8.0) with proportion of 1:100 and filtered by a 0.22 μm membrane. The preparation of apple sample was the same as that of the Chinese cabbage sample.

3. Results

3.1. Design strategy

As shown in Fig. S1, the 54-mer sequence of the ICP aptamer was split into two shorter fragments, wherein A-24 contains 24 bases and A-30 contains 30 bases. As the split site on the aptamer was located far