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Magnetic-assisted aptamer-based fluorescent assay for allergen detection in food matrix

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ABSTRACT

Herein, a facile and versatile label-free aptamer-based fluorescent assay for simple and sensitive detection of food allergen in food matrix is developed based on the functionalized magnetic nanoparticles (MNPs) as separation carrier and commercially available OliGreen dye as signal probe. First, the aptamer hybridized with capture probe that was conjugated on the surface of MNPs to form aptamer-MNPs complexes as detection probes, which undergo conformational change upon interaction with target analytes, resulting in the release of aptamer from the surface of MNPs. So the released aptamer in the supernatant produced a significant fluorescence enhancement signal by introducing the OliGreen dye based on its ultrasensitive and specific fluorescence enhancement upon binding to ssDNA. As proof-of concept, this proposed strategy was applied to quantitative detection of tropomyosin, one of the major allergen found in shellfish. Under optimal conditions, the linear range was from 0.4 to 5 $\mu g\,mL^{-1}$ ($R^2 = 0.996$) with a low limit of detection calculated to be 77 $ng\,mL^{-1}$. Moreover, the aptamer-based assay had remarkable selectivity and was successfully applied in the detection of tropomyosin in food matrix. Due to its simple design, easy operation and high sensitivity and selectivity, the proposed aptamer assay could expect to expand applications in various allergens detection by changing corresponding aptamers on the detection probe.

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1. Introduction

Food allergies involve different hypersensitivity reactions, which are mediated by the immune system in human body exposed to the harmful allergens (mostly proteins) [1]. To date, food allergy has no effective medical treatment and the only possible prevention relies heavily on a complete avoidance of the specific allergen-containing food [2]. These present a vital food safety issue for individuals affected by severe, life-threatening food allergies. Hence, high sensitive and selective allergen detection methods are of utmost importance in food allergy diagnosis and food-allergic consumers' protection.

Up to date, various enzyme-linked immunosorbent assay (ELISA) approaches that are all depended on the immuno-interaction have been described and recommended for allergen detection due to their specificity and sensitivity [3–5]. Generally, the quantification is based on the colored product that is produced by an enzyme that is coupled to the "detection" antibody. However, purification within SDS-PAGE or affinity tags (e.g. biotin) is usually needed to avoid false positives due to the cross-reactivity of antibodies with other proteins in the sample [6]. Furthermore, the production of antibodies is expensive and time-consuming. On the other hand, the stability of antibodies has heavily limited its utilization for a long time. Compared to these detection modalities, aptamer-based assays offered lower sample requirements, higher sensitivity and specificity.

Recently, aptamer-based assays integrated with a large number of new nanomaterials, such as quantum dots (Qdots) [7,8], gold nanoparticles [9,10], graphene oxides [11,12], and magnetic

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nanoparticles (MNPs) [13,14], have been applied to the detection of allergens. Such methods have exhibited remarkable sensitivity and selectivity based on the specific recognition ability of aptamer and signal amplification strategy of nanomaterials. For instance, Weng and Neethirajan [7] developed a microfluidic system integrated with an aptamer/Qdots-functionalized graphene oxide (GO) biosensor for simple, rapid, and sensitive detection of peanut allergen and their system showed remarkable sensitivity and selectivity with detection limits of 56 ng mL^{-1}. Among which, DNA aptamer can be covalently bound to the surface of MNPs with the effect of chemical coupling attributing to the flexibility and simplicity of MMPs functionalization. Moreover, magnetic separation is easy operation and will effectively reduce or eliminate the interferences from complex matrix [15]. Therefore, MNPs is an attractive option to set up bioassays and thus a higher sensitivity could be provided by magnetic separation.

In order to reach more sensitive detection, various novel aptasensors have been developed owing to the intrinsic properties of aptamers, such as high flexibility of structure and convenience in the design of their structure. Complementary sequences can easily modulate the formation of aptamers and this aptamer-complementary DNA complex can be easily destroyed by competitive interaction among aptamer, target, and complementary DNA. This unique property of aptamers resulted in many new novel methods in signal generation formats, which were widely applied in various targets detection [16–19]. For example, Li et al. [16] developed an ultrasensitive chemiluminescence biosensor for the detection of protein based on the functionalized magnetic microparticles and the hybridization chain reaction (HCR). Despite the progress made in development and application of these aptamer-based assays for analytical applications, the majority of the strategies developed require nanomaterials or fluorophores to label aptamers, which severely restrict their applications in bioanalysis due to the modification of aptamers is expensive [20,21] and the fluorescent and/or quenching molecules may even reduce the binding properties of aptamers [22–24]. In addition, assays based on this unique property for food allergen detection are scarcely reported especially for those based on label-free aptamer.

Tropomyosin is the major heat-stable and cross-reactive allergen found in shellfish, such as shrimp, crab and lobster [25–27]. With the increased consumption of shellfish across many countries, adverse reactions to shellfish have heavily affected people's quality of life [28] and represented a major concern [29]. Given the importance of tropomyosin in predicting shellfish allergies [30], development of valid assays for the detection of tropomyosin in food products would be greatly in demand. In our previous work [31], we have identified DNA aptamers selectively recognizing tropomyosin with high affinity. Subsequently, Amouzadeh Tabrizi et al. [32] have employed our identified aptamer to fabricate a high sensitive visible light-driven photoelectrochemical biosensor for tropomyosin detection. However, this aptasensor report on the detection of tropomyosin in human serum, which cannot meet the testing requirements in food. Therefore, extension of aptamer-based approaches to allow detection of tropomyosin in food matrix would be broadly useful.

Inspired by these concepts, in this study, we developed a simple and universal label-free aptamer-based strategy for tropomyosin detection in food matrix by using commercially available OliGreen dye as signal probe and magnetic nanoparticles as separation carrier. Comparing with the other reported strategies for tropomyosin detection, there were just label-free aptamer, magnetic nanoparticles and OliGreen dye involved in our sensitive analytical microsystems, which offered a really simple and low-cost aptamer-based strategy for tropomyosin detection. To the best of our knowledge, no magnetic-assisted aptamer-based fluorescent assay for tropomyosin detection has yet been published in literatures. In addition, the assay can be extended to detect various targets such as antibiotics, toxins and other allergens, just by changing the aptamers on the detection probe.

2. Materials and methods

2.1. Materials and reagents

The amino-functionalized MNPs (10–30 nm, 25% in H_2O, Macklin) were purchased from Beijing John Long Technology Co., Ltd. (Beijing, China). Sulfosuccinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (Sulfo-SMCC) was purchased from Shanghai Aladdin biochemical technology Co., Ltd. (Shanghai, China). Quant-iT^{TM} OliGreen ssDNA reagent (Molecular Probes, Invitrogen) was purchased from Thermo Fisher Scientific (China) co., Ltd (Shanghai, China). Shrimp tropomyosin (*Penaeus Vanmamei*) was purchased from Shanghai Anyan Biotechnology Co., Ltd. (Shanghai, China). Bovine Serum Albumin (BSA) and streptavidin were purchased from Sigma-Aldrich Co., Ltd. (Beijing, China). β-conglycinin was obtained from Shanghai Bangyi Biotechnology Co., Ltd. (Shanghai, China). Lysozyme and myosin were purchased from Beijing Huayueyang Biotechnology Co., Ltd. (Beijing, China). All chemicals for preparing the buffers and solutions were of analytical grade and obtained from Shanghai CIVI Chemical technology Co., Ltd. (Shanghai, China). The solutions were prepared with ultra-high purity water from a Millipore water purification system. All oligonucleotides purified by HPLC were chemically synthesized by Sangon Biotechnology Co., Ltd. (Shanghai, China). The sequences of the tropomyosin aptamer [31] and capture probe are as follows:

Tropomyosin aptamer:

5′-TACTAACGGTACAAGCTACCAGGCCGCCAACGTTGACC-TAGAAGCACTGCCAGACCCGAACGTTGACCTAGAAGC-3′

Capture probe: 5′-HS-C6- GCTTCTAGGTCAACGTT-3′

2.2. Preparation of magnetic aptamer-immobilized detection probe

The preparation of magnetic aptamer-immobilized detection probe is illustrated in Scheme 1. Firstly, capture probe functionalized MNPs were synthesized by the crosslinking reaction of amino-functionalized MNPs and sulfhydryl-labeled capture probe. Generally, 100 μL of commercialized amino-functionalized MNPs suspension (25% in H_2O) were washed three times with the same volume PBS buffer (Na_2HPO_4 10 mM, KH_2PO_4 1.5 mM, NaCl 150 mM, KCl 2.7 mM, pH 7.5) and dispersed into 5 mL of PBS buffer. 100 μL of Sulfo-SMCC in DMSO (100 mg mL^{-1}) was added to the washed amino-functionalized MNPs, and the mixture was reacted for 1 h at room temperature with periodic mixing. Then, the activated MNPs were immediately isolated with a magnet and washed three times by PBS buffer. Subsequently, the purified MNPs were mixed at the desired molar ratio with sulfhydryl-containing capture probe in 5 mL of PBS buffer, and reacted for 24 h at room temperature with periodic mixing. The resulting capture probe functionalized MNPs were isolated with a magnet and washed three times by binding buffer (Tris–HCl 10 mM, NaCl 150 mM, KCl 10 mM, $MgCl_2$ 2.5 mM, pH 7.2). The capture probe functionalized MNPs was dispersed into 1.25 mL of binding buffer and saved at 4 °C before to use.

Secondly, the magnetic aptamer-immobilized detection probe was prepared based on the hybridization of aptamer and capture probe. In brief, 2 mL of capture probe functionalized MNPs (0.5% in binding buffer) was added 100 μL of 100 μM tropomyosin aptamer, and the mixture was heated at 95 °C for 5 min, and slowly cooled to room temperature for almost 1 h. The resulting magnetic detection probe was isolated with a magnet and washed three times by bind-

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