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Short communication

Organophosphorus pesticides detection using broad-specific single-stranded DNA based fluorescence polarization aptamer assay

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ABSTRACT

An approach is developed to detect the organophosphorus pesticides via competitive binding to a recombinant broad-specificity DNA aptamer with a molecular beacon (MB), the binding of the MB to the aptamer results in the activation of a fluorescent signal, which can be measured for pesticide quantification. Aptamers selected via the Systematic Evolution of Ligands by Exponential Enrichment (SELEX) were structurally modified and truncated to narrow down the binding region of the target, which indicated that loops of the aptamer contributed different functions for different chemical recognition. Thereafter, a variant fused by two different minimum functional structures, was clarified with broad specificity and increased affinity. Further molecular docking and molecular dynamics simulations was conducted to understand the molecular interaction between DNA structure and chemicals. 3D modeling revealed a hot spot area formed by 3 binding sites, forces including hydrogen bonds and van der Waals interactions appear to play a significant role in enabling and stabilizing the binding of chemicals. Finally, an engineered aptamer based approach for the detection of organophosphorus pesticides was successfully applied in a test using a real sample, the limit of quantification (LOQ) for phorate, profenofos, isocarbophos, and omethoate reached 19.2, 13.4, 17.2, and 23.4 nM (0.005 mg L^{-1}), respectively.

1. Introduction

The concern related to pesticide residues in the diet with regard to possible health risks is widespread and has increased in recent years, particularly related to highly poisonous organophosphorus pesticides, such as phorate, profenofos, isocarbophos, and omethoate. Any possible residues of or contamination by these pesticides could have serious consequences for human health. Therefore, the need for a high-throughput screening approach with a predetermined selectivity and affinity for target analytes is greater than ever.

As bio-elements used for recognition, oligonucleotides aptamers exhibit many advantages over other bio-elements, such as their small size, allowing easy synthesis, easy-to-modify, low immunogenic, and chemically stability, and are regarded as promising substitutes for antigen-antibody reactions. Molecular beacon (MB) is a nucleic acid motif with a hairpin-shaped structure that has one fluorophore and one non-fluorescent quencher covalently linked to each end of its stem, resulting in low fluorescence, and the loop sequence is complementary to the target sequence as a probe (Tyagi and Kramer, 1996; Yamamoto et al., 2000). When the MB encounters a target molecule, the binding of the target disrupts the stem and separates the fluorophore from the quencher, leading to a fluorescence signal. Tuerk and Gold (1990) accomplished the selection of T4 DNA polymerase-binding sequences from an RNA pool and referred to this selection procedure as Systematic Evolution of Ligands by Exponential Enrichment (SELEX). Moreover, with the development of SELEX technology modification, and post-SELEX optimization, the aptamer can be given additional properties, such as the use of chemically produced or modified oligonucleotide libraries to select aptamers with desired features (Green et al., 1995), and the use of chemical modification or base modification can introduce new features into the aptamers (Gold et al., 1995), sometimes aptamer truncations even result in raised affinity (Burke et al., 1996; Ruckman et al., 1998), and fused aptamers could combine different features (Yoshida et al., 2006). But the problem of the requirement for family chemical rapid screening detection remains. For example, family chemical organophosphorus pesticides, which have the toxic effect and chemical structure in common, some of them maybe too low to be detected, but all of them may cause cumulative (additional joint) adverse health effects, so broad-specificity based screening approach with predetermined selectivity is essential to provide an attention for

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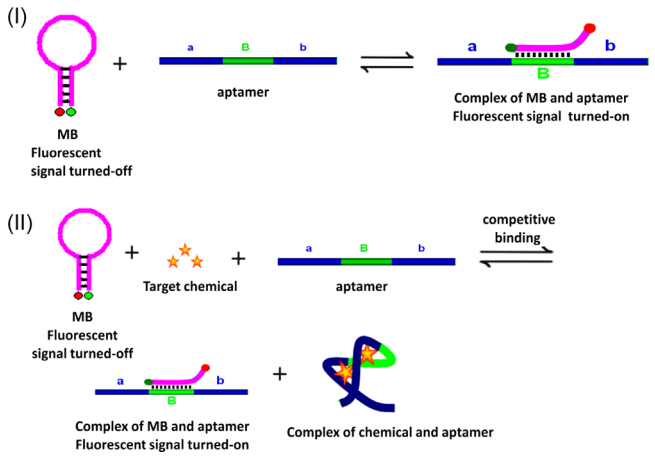
human health benefit. It is possible now to analyze co-existing structures within a population of aptamers with identical sequence (Schroder et al., 1998), but no research has been reported for the simultaneous detection of co-existing chemicals with one broad-specificity aptamer.

Currently, significant interest is focused on development of small molecule aptamers owing to their applications in biosensing, diagnostics, and therapeutics. In spite of the very encouraging promises and great potential for the diverse applications, relatively few aptamers that bind to small molecules have been reported, and methodologies to enhance and broaden their functions by expanding chemical repertories have barely been examined. In a previous study by our group (Wang et al., 2012), aptamers were selected from an immobilized, random single-stranded DNA (ssDNA) library using the SELEX technique, which presented broad specificity for four organophosphorus pesticides recognition. These ssDNAs included primer-binding sequences at both ends and a middle constant sequence, with two random regions of the ssDNA located between the constant sequences; constant middle region of the oligonucleotide library that was used to immobilize the ssDNA library by a partly complementary sequence during the selection of the aptamer (Nutiu and Li, 2005). Herein, in this study, further structural modifications of the aptamers were designed and synthesized with the aim of better characterizing their affinity and specificity for pesticides. Subsequently, a novel competition approach based on a broad-specificity aptamer with increased affinity was explored for simultaneous detection of these substances (Scheme 1), and was successfully exploited using real test sample following sample extraction and cleanup. To our knowledge, this study represents the first report of multi-analysis of organophosphorus pesticides using engineered broad-specificity ssDNA aptamers with a fluorescence signal that is turned on by MB competitive binding, and the developed approach could achieve low-level detection.

2. Experimental

The details of experiments are given in Supplementary material (SI). In brief, aptamer and variants were chemically synthesized; MB 5'-FAM-CTGCACAAGAATCGCTGCAG-DABCYL-3' was used for the competitive binding to aptamers, and the fluorescence intensity changes of MB binding to ssDNA aptamer was calculated as inhibition ratio (IR). Using Visual Molecular Dynamics (VMD) (Hsin et al., 2008; Humphrey et al., 1996) and NAnoscale Molecular Dynamics (NAMD) (Phillips et al., 2005) for aptamer structure modeling and docking, the composited aptamer–ligand structure was energy minimized and equilibrated according to published methods (Bruno et al., 2008), and the Molecular Mechanics – Generalized Born Surface Area (MM – GBSA) method implemented in AmberTool12 was used to calculate the binding energy.



Scheme 1. Strategy for the determination of pesticides: "a" and "b" are the regions of the aptamer derived from the random region of library used during the selection of the aptamer; B present the constant sequences, which can complementary binding to MB. (I) present the control model with chemical absence and (II) present the competition model with chemical presence; compared with the condition (I), the turn-on fluorescent signal in condition (II) will be decreased by the chemical competitively binding to aptamer, and the changes in fluorescence is associated with the changes in the concentration of the chemical.

3. Results and discussion

3.1. Optimization of assay conditions

The details of assay conditions are given in Supplementary material (SI). The strategy for competitive assay development is to incubate the aptamer with the chemical first, and then the MB is added to turn on the signal for detection, and the homogeneous assay is performed at 25 °C for 50 min in aqueous solution of 50 mM NaCl, 10 mM KCl, 10 mM $MgCl_2$, and 50 mM Tris/HCl, pH 8.0, with a final acetone content of below 1%.

3.2. Structural modification and characterization

In principle, an aptamer can specifically recognize and bind to a unique ligand, leading to a structural change of an aptamer, which are oligonucleotides (RNA or ssDNA) binding to their target with high selectivity and sensitivity because of their three-dimensional shape, not only by their sequence. DNA aptamers have the capacity to form distinct secondary and tertiary structures that can bind targets with considerable affinity and specificity, structures of aptamer complexes reveal the key molecular interactions conferring specificity to the aptamer–ligand association, including the precise stacking of flat moieties, specific hydrogen bonding, and molecular shape complementarity (Hermann et al., 2000), the stem-loop shows a correlation between sequence structure and function (Nonin-Lecomte et al., 2001). Therefore, certain characteristics of an aptamer can be structurally engineered to create a series of variants or segments, and some may display increased affinity or specificity under multi-characterization, even at the minimum functional molecular size. We have structurally truncated, spliced, and chemically synthesized aptamer variants and segments with the aim of employing a minimum functional structure to examine the correlation between sequence structure and broad-specificity function, and some sequences have been chained together with the intent of extending this broad specificity. The sequences and secondary structures are presented in Supplementary table S2 and Fig. S8. The affinity and specificity of the variants were tested using the targets, which show various dramatically changing affinities and specificities according to the different design strategies employed (Fig. 1).

For the parent aptamer SS2-55, the terminal stem of the stem-loop structure was first shortened by cutting through loop 2–4, with two base pairs of the adjacent loop mutated to induce a destabilized conformation, designated SS2-C-44. This design strategy caused a decrease in the affinity and specificity toward chemicals, particularly the pesticide omethoate, for which only a 5.81% affinity was retained, which implying that the terminal stem and loop 2–4 may play a critical structural role in the broad recognition of chemicals by the aptamer and in specific binding to omethoate, with phorate representing an exception in this case. Next, loop 2–3 was cut, and the remaining SS2-C-29 segment lost almost all affinity and specificity for phorate but retained affinity and specificity for the other chemicals compared with SS2-C-44,