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Quantum dot-DNA aptamer conjugates coupled with capillary electrophoresis: A universal strategy for ratiometric detection of organophosphorus pesticides

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ABSTRACT

Based on the highly sensitivity and stable-fluorescence of water-soluble CdTe/CdS core–shell quantum dots (QDs) with broad-specificity DNA aptamers, a novel ratiometric detection strategy was proposed for the sensitive detection of organophosphorus pesticides by capillary electrophoresis with laser-induced fluorescence (CE–LIF). The as-prepared QDs were first conjugated with the amino-modified oligonucleotide (AMO) by amidation reaction, which is partial complementary to the DNA aptamer of organophosphorus pesticides. Then QD-labeled AMO (QD-AMO) was incubated with the DNA aptamer to form QD-AMO–aptamer duplex. When the target organophosphorus pesticides were added, they could specifically bind the DNA aptamer, leading to the cleavage of QD-AMO–aptamer duplex, accompany with the release of QD-AMO. As a result, the ratio of peak height between QD-AMO and QD-AMO–aptamer duplex changed in the detection process of CE–LIF. This strategy was subsequently applied for the detection of phorate, profenofos, isocarbophos, and omethoate with the detection limits of 0.20, 0.10, 0.17, and 0.23 μM, respectively. This is the first report about using QDs as the signal indicators for organophosphorus pesticides detection based on broad-specificity DNA aptamers by CE–LIF, thus contributing to extend the scope of application of QDs in different fields. The proposed method has great potential to be a universal strategy for rapid detection of aptamer–specific small molecule targets by simply changing the types of aptamer sequences.

1. Introduction

Organophosphorus pesticides (OPs) are widely used throughout the world to prevent crop losses and have attracted a great deal of attention. They are a class of chemicals that generally act as cholinesterase inhibitors and have been commonly used in agriculture due to their high efficiency as insecticides [1,2]. Long-term exposure to OPs-polluted environment will do great harm to people's health.

The current methods for qualitative and quantitative analysis of OPs are mainly based on gas chromatography (GC) [3], high performance liquid chromatography (HPLC) [4], fluorescence [5] and electrochemistry [6]. They are accurate and sensitive. However, some of them are laboratory-based, time-consuming, or required expensive instruments and high consumption of reagents. In addition, pre-concentration or extraction steps are usually necessary before analysis. So it is necessary to develop a rapid, sensitive, and effective method for the detection of OPs residues in crops or environment samples.

As an effective separation technique, the capillary electrophoresis (CE) is very promising to be applied to pesticide analysis because of its advantages, such as higher separation efficiency, high analysis speed, and very small consumption of expensive reagents and toxic solvents. Affinity probe capillary electrophoresis (APCE) refers to a collection of techniques in which high-affinity binding is used in conjunction with CE separation to determine analytes. Coupled with laser-induced fluorescence (LIF) detection, it has demonstrated potential for rapid determination of trace levels of analyte in complex mixtures [7,8]. CE–LIF-based affinity assays can be performed in either a competitive or a noncompetitive (direct) format. The competitive format is described more frequently because it offers (i) a significant change in the mobility of any small analyte upon binding to a large molecule and (ii) it facilitates the preparation of a uniform tracer.

Generally, the analytes need to be labeled by fluorescent derivative reagents before LIF analysis because most substances are not fluorescent [9–12]. However, most common organic

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fluorophores, such as fluorescein isothiocyanate, only emit one fluorescence signal and have low quantum yield in the visible light range. In addition, they are unstable and prone to photobleaching [13]. Compared with traditional fluorescent substances, luminescent quantum dots (QDs) have many unique optical properties, such as high fluorescence, good photochemical stability, and tunable excitation/fluorescence wavelength at different particle sizes [14–16]. They emerge as alternative or complementary tools to the organic fluorophores currently used in various fields [12]. So they can be used as suitable photochemically stable fluorescence agents for LIF detection. Besides that, QDs are easy to synthesize with mature methods and need lower cost, shorter derivative time and better stability. Linked with biorecognition molecules (e.g., proteins, peptides and DNA), QDs have been widely used in biological and medical fields, such as immunoassay [17,18], DNA hybridization [19,20], cell imaging [21–26], etc. Therefore, QDs are competitive alternates as fluorescence labels in many fields and QDs–labeled aptamers have displayed great potential in CE–LIF for rapid determination of trace levels of analyte in complex mixtures [27,28].

Currently, significant interest is focused on development of small molecule aptamers, but relatively few aptamer–target complexes employed in CE–LIF have been reported [19,25]. The aptamer–target complexes are stable in binding buffer, but a potential complex dissociation may occur during the course of electrophoresis [29] and hardly exist in non-equilibrium CE separation. The affinity may be weaker in CE because of the lower ionic strength of binding buffer. Furthermore, the binding interaction is not at equilibrium, which was the nature of this CE-based assay [25]. These may have influence on the accuracy of the analytical results. To overcome this problem, a universal ratiometric detection strategy was proposed in this study by changing the position of AMO and aptamer. It detects the free QD-AMO and/or QD-AMO–aptamer duplex instead of aptamer–target complex, which could reduce the influence made by the dissociation of aptamer–target complex. This universal ratiometric method is matrix-free compared with other CE separation modes. It can improve signal-to-noise ratio and avoid the systemic error effectively, which is more accurate than single signal detection.

In this work, the universal ratiometric detection approach based on CdTe/CdS core/shell QDs coupled broad-specificity aptamer with increased affinity was explored for simultaneous detection of organophosphorus pesticides (shown in Fig. 1), and was successfully applied to real sample. To the best of our knowledge, this is the first report of multi-analysis of organophosphorus pesticides using QDs and broad-specificity DNA aptamers by CE–LIF. The ratiometric detection was essentially realized by specific binding between pesticides and aptamer, which induced the ratio of peak height changes, thus high sensitivity detection could be achieved by the amplified signal readout through simultaneous readings of two channels. Here, by combining the advantages of CE–LIF-based affinity assays, good stability and high fluorescence of QDs and accuracy of ratiometric detection, the developed method paves a new avenue for designing universal strategy for ratiometric detection of organophosphorus pesticides.

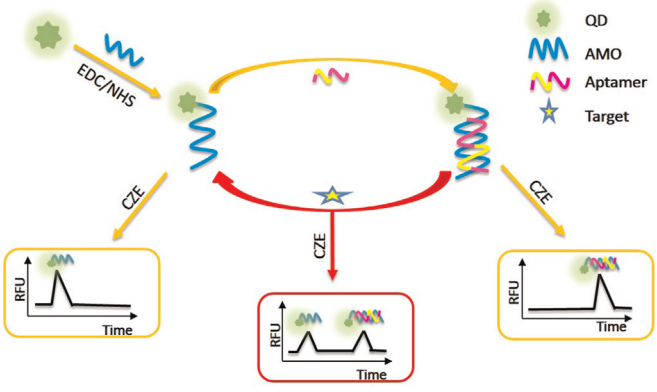

Fig.1. Schematic representation of the structure-switch aptamer principle and the ratiometric detection of organophosphorus pesticides by capillary electrophoresis with laser-induced fluorescence (CE–LIF).

2. Experimental

2.1. Chemicals and Materials

The DNA aptamer (5′-AGCTTGCTGCAGCGATTCTTGATCGCCA-CAGAGCT-3′) and its complementary strand (AMO: 5′-NH_2-A_{10} -CAAGAATCGCTGCAG-3′) were synthesized by Sangon Biotech Co. Ltd. (Shanghai, China). Phorate, profenofos, isocarbophos and omethoatewere purchased from J&K (Shanghai, China). The mercaptopropionic acid (MPA), 1-ethyl-3-(3-dimethylamino propyl) carbodiimide (EDC) and N-hydroxysulfo-succinimide (Sulfo-NHS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were of analytical purity and obtained from Shanghai Chemical Reagent Company (Shanghai, China). Deionized water (DI water) was purified in a Milli-Q water purification system Millipore (Bedford, MA, USA). Aptamer solutions were purified with Milli-Q A10 filtration systems (Millipore, Billerica, MA, USA).

2.2. Apparatus

The capillary electrophoresis equipment was constructed in our laboratory. TriSepTM-2100-LIF detector (Unimicro Technologies, USA) was coupled with CE. Electrophoresis in the capillary was driven by a $\pm$30 kV high voltage with supplier (Shanghai Institute of Applied Physics, China). LIF detection was used with an excitation wavelength of 473 nm and an emission wavelength of 520 nm. The fluorescence and the absorption spectra were recorded with a Hitachi F-7000 fluorescence spectrophotometer (Tokyo, Japan) and a Shimadzu UV-1800 spectrophotometer (Tokyo, Japan), respectively.

2.3. Preparation of quantum dots

Thiourea was used as the source of sulfur and was added directly to the original MPA-capped CdTe reaction solution, water-soluble CdTe/CdS core/shell QD capped with MPA were prepared according to the procedure reported in the literature [30]. Briefly, 50.8 mg of tellurium powder and 37.8 mg of $NaBH_4$ were loaded in a 25 ml two-necked flask under N_2 flow. Then 10.0 ml of distilled water was added through a syringe. The reaction mixture was heated at 80 °C for 30 min under N_2 flow to get a deep red clear solution, after that the NaHTe solution were obtained. Then 0.2 mmol of Cd solution, and 0.4 mmol of MPA solution were mixed in a 40 ml solution and the pH of the solution was adjusted to 10.0 by 1.0 M NaOH solution. Under stirring, 2.0 mL of freshly prepared NaHTe solution was added through a syringe into the Cd precursor solution at room temperature. So the ratio of Cd:MPA:Te in the reaction solution was 1:2:0.2. Then the reaction mixture was refluxed at 100 °C under N_2 protection. The CdS shell was grown around the CdTe core seeds in a solution with the following concentration ratios: thiourea:Cd:Te:MPA=4:1:0.2:2 (Fig. S1).

2.4. Preparation of QDs-AMO/aptamer conjugates

For QD-AMO preparation, the –COOH group of QDs was first activated by mixing with EDC and Sulfo-NHS in phosphate-buffered saline (PBS: 10 mM phosphate, pH 7.4, 137 mM NaCl, and