Selection of Pesticide Residue Aptamers and Sensing Detection Technologies

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ABSTRACT

Pesticide small molecules, especially organophosphorus pesticides, carbamate pesticides, and bipyridyl pesticides, are effective means for weed and pest control to improve crop yield and quality. However, long term use has led to their accumulation in soil, crops, and wastewater runoff, causing significant negative impacts such as environmental pollution, global cross contamination of agricultural products, and an increase in human and animal diseases. Therefore, developing rapid detection technologies and devices for pesticide small molecules to achieve efficient and strict monitoring of pesticide residue pollution is a major strategic requirement for ensuring food and environmental safety.

Aptamers are short single stranded oligonucleotides (RNA or DNA) obtained through the Systematic Evolution of Ligands by Exponential enrichment (SELEX) technology. They can specifically recognize small molecules. Compared with antibodies or enzymes, aptamers have the advantages of better stability, low molecular weight, easy modification, and low cost, making them excellent candidates for developing aptamer sensors for detecting pesticide residues.

Therefore, in this study, the SELEX technology with streptavidin magnetic beads as the medium was adopted, and aptamer probes capable of specifically capturing pesticide small molecules were successfully obtained, providing new recognition materials for the convenient and rapid detection of pesticide residues. In addition, highly sensitive, rapid, and simple aptamer sensors were constructed by combining fluorescence and colorimetry methods respectively for the detection of pesticide small molecules. This provides theoretical and experimental basis for on site rapid detection of pesticide residues in various scenarios by agricultural product quality inspection stations, fruit and vegetable production bases, markets, and environmental supervision departments, as well as for the further development of detection kits for food and environmental safety hazards. The main contents of this study are as follows:

SELEX Screening Technology for Pesticide Small Molecule Aptamers Based on Streptavidin Magnetic Beads

A technology suitable for the screening of nucleic acid aptamers for small pesticide molecules was established, including the design of random libraries and primers, the immobilization and enrichment of libraries, PCR amplification, and the preparation and purification of single stranded DNA. The streptavidin magnetic bead method was used to immobilize the library, which had high immobilization efficiency, and the eluent could be quickly obtained through magnetic separation. The emulsion PCR was employed to amplify the library, which reduced the preference problem in conventional PCR amplification. The denaturing PAGE electrophoresis was used to separate

single stranded DNA, and n butanol was used to concentrate the products. This method had low cost, and the separation effect of single stranded DNA was better than that of the asymmetric PCR amplification method. After 7 9 rounds of screening, large capacity nucleic acid aptamer libraries with good enrichment for four pesticides, carbaryl, chlorpyrifos, carbofuran, and paraquat, were successfully obtained. Subsequently, cloning sequencing or high throughput sequencing analysis was carried out, and candidate nucleic acid aptamers Apta3, CBF 5, Chl 1, and PQ 5 with the strongest binding ability to each pesticide target were verified. These provide new biological recognition materials for the efficient detection of four small pesticide residue molecules in food and the environment.

Construction of a turn-on fluorescent aptamer sensor and its application in the detection of carbaryl pesticides

To achieve the rapid detection of small pesticide molecules, this study constructs a turn-on type fluorescent aptamer sensor. First, the carbaryl aptamer Apta3 obtained through screening was trimmed and optimized to obtain Apta3S, which was used as the recognition probe. Subsequently, a fluorescent signal group was modified on the aptamer, and a short-chain modified quenching group was designed, so that the fluorescent signal of the aptamer was pre quenched. This fluorescence detection method has good stability and strong specificity. It only contains two probes, which makes the operation simple and the cost low. Moreover, the pre quenching of fluorescence results in a low background value of the detection platform, making it highly practical. The constructed sensor has achieved the quantitative detection of carbaryl pesticides, with a detection limit of 15.23 nmol/L, meeting the requirements of the national standard [Groundwater Quality Standard (Class III)].

Construction of a DNA-Walker Fluorescent Aptamer Sensor and Its Application in the Detection of Carbofuran Pesticide

To improve the sensitivity weakness of the turn-on fluorescent aptamer sensor, we constructed a fluorescent aptamer sensor based on the DNA-Walker signal amplification technology. The constructed sensor consists of three parts: the swing arm probe, the signal probe, and the aptamer. As a recognition probe, the aptamer can specifically recognize carbofuran pesticide. After the carbofuran target binds to the aptamer, according to the high specificity of the Watson-Crick base pairing principle, the swing arm probe immediately binds to the signal probe. Driven by the enzyme, it initiates enzymatic cleavage to release the fluorescent signal and drives the swing arm probe to move autonomously along the set oligonucleotide track, subsequently releasing more fluorescent signals to achieve the effect of signal amplification. The designed DNA-Walker signal amplification technology drives the swing arm probe to move through the enzyme, avoiding the problem of detachment during the process of repeatedly carrying multiple DNAs to drive the swing arm probe. In addition, using magnetic beads as the carrier and recovering the solution

through magnetic separation, the operation is simple and efficient. This sensor was applied to the quantitative analysis of carbofuran pesticide, and the sensitivity reached 72.64 pM, meeting the requirements of the national standards [Groundwater Quality Standard (Class I)] and [National Food Safety Standard].

Construction of a label-free colorimetric aptasensor based on AuNPs and its application in the detection of chlorpyrifos pesticides

Colorimetric analysis has unique advantages such as simple fabrication, independence from specialized equipment, good portability, low cost, and strong visual detection ability. It is another most commonly used detection method in optical analysis. In order to simplify the operation steps and develop a method for rapid on site detection, this study constructs a label free aptamer colorimetric sensor based on AuNPs.

Due to the unique optical absorption and scattering properties of AuNPs, their aggregation can induce inter particle plasmon coupling, leading to local surface plasmon resonance shifts and visible color changes in the nanoparticle solution, which shows great potential for colorimetric detection applications. In this study, aptamers are used as recognition probes and are wrapped on the surface of AuNPs through electrostatic interaction, which can act as a stabilizer to prevent the aggregation of AuNPs in salt solutions.

When the chlorpyrifos target is present, it is specifically captured by the aptamer. Subsequently, the AuNPs are exposed to a high salt solution, and the salt induced aggregation of AuNPs occurs to varying degrees, causing the solution to gradually change from wine red to blue purple. The AuNPs used in this study do not require additional modification, and this method has fewer steps, is simple to operate, and has low cost. It can quantitatively detect the chlorpyrifos target with a detection limit of 14.46 nmol/L, meeting the requirements of the national standards [Groundwater Quality Standard (Class II)] and [National Food Safety Standard].

In addition, the detection of actual samples shows that this sensor has good stability and strong specificity in organic solvents or solutions containing matrix components.

Establishment of a colorimetric lateral flow sensor based on AuNPs-aptamer and its application in the detection of paraquat pesticides

Lateral flow devices are one of the most widely used point-of-care testing (POCT) tools, featuring simplicity, ease of operation, rapidity, miniaturization, and low cost. To achieve on-site and rapid detection of small pesticide molecules, we developed a colorimetric lateral flow assay based on AuNPs-aptamer.

First, probes containing aptamer sequences were conjugated to AuNPs, and probes complementary to the aptamers were sprayed on the test line. Paraquat pesticides compete with the probes on the test line for aptamers. When there is no paraquat pesticide in the test sample, the AuNPs-conjugated aptamers bind to the probes on the test line, resulting in a strong signal on the

test line. When paraquat pesticide is present in the test sample, the AuNPs-conjugated aptamers bind to the paraquat pesticide, leading to a weak or no signal on the test line. The more paraquat pesticide is present in the test sample, the weaker the signal on the test line.

The developed colorimetric lateral flow assay based on AuNPs-aptamer has good reproducibility and low cost. The prepared test strips can be stored sealed for up to six months and still remain usable. Detection of real samples shows that this sensor can specifically detect paraquat pesticides with a detection limit of 0.8 μmol/L. It can serve as a practical tool for on-site and rapid detection of pesticide residues in agricultural product quality inspection stations, fruit and vegetable production bases, markets, and environmental supervision departments.

CONTENTS

CHAPTER 1

Introduction

1.1 Overview of the Current Situation of Food and Environmental Safety

Food is the primary necessity for humans, and agriculture is regarded as the main means of livelihood for more than 50% of the world's population. Moreover, according to the current population growth trend, the global population is expected to exceed 10 billion by 2050 [1–3], and the global demand for food will continue to increase. In the entire food industry chain, factors harmful to human health can be introduced through various channels such as the production, processing, storage, and circulation of agricultural and livestock raw materials. These risk factors include various chemical and microbial sources, such as pesticide and veterinary drug residues, pathogenic organisms, biotoxins, and organic pollutants [4, 5]. China is a major country in food production and consumption, and food safety has always been a key issue of concern in China. The newly revised Food Safety Law of the People's Republic of China clearly requires that food safety monitoring should cover the entire industrial chain of production, processing, storage, and transportation. Using rapid detection technology to conduct spot checks on each link is not only an important means to implement the Food Safety Law but also widely welcomed by front line food safety monitoring departments and enterprises. Therefore, researching and developing rapid detection technologies and products is a major strategic need to ensure food safety in China.

In addition, with the rapid development of industrialization and urbanization over the decades, environmental problems such as the pollution of air, water, and other natural resources have become major concerns globally. Some of the main anthropogenic sources contributing to environmental deterioration come from industrial and agricultural activities, such as improper solid waste disposal and the bio accumulation of toxic waste, the extensive use of pesticides and synthetic fertilizers, and the use of biological warfare agents [6]. Due to the toxicity of these pollutants, humans are often exposed to various adverse health effects, mainly through the ingestion of contaminated food and drinking water, as well as the inhalation of ambient air containing high concentrations of pollutants [7]. Therefore, it is of great significance to develop reliable, highly sensitive, user friendly, and low cost detection methods for continuous monitoring and detection of pollutants in different media.

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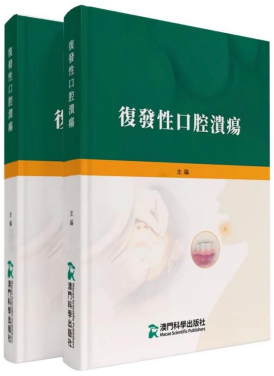

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- 内分泌系统疾病 Endocrine system disease

- 实用小儿内科学 Practical pediatric internal medicine
- 消化系统疾病诊疗学 Diagnosis and treatment of digestive system diseases
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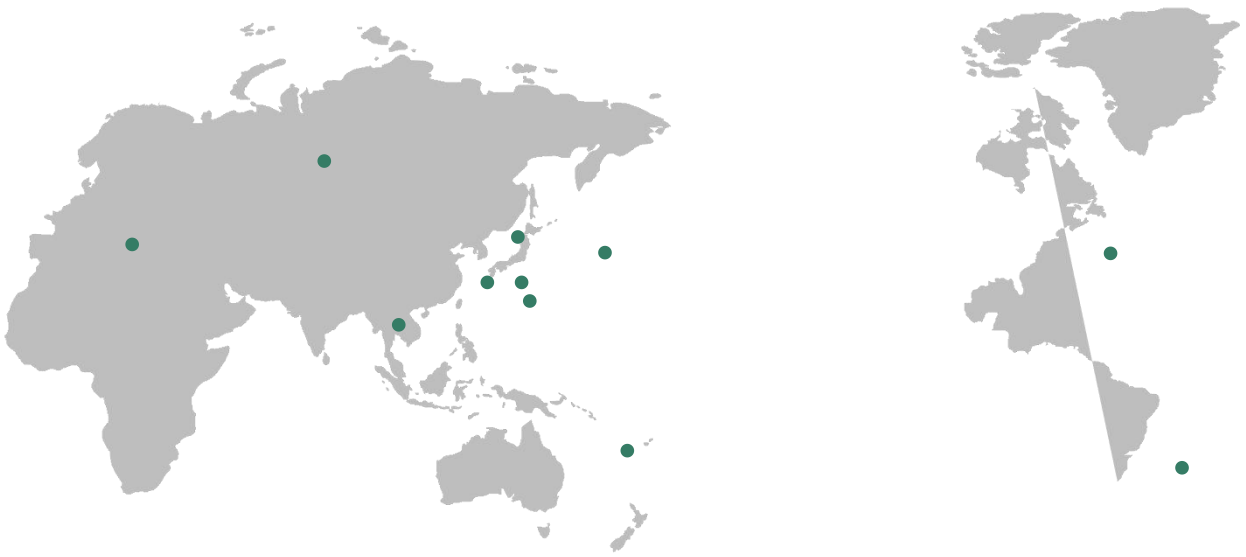

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