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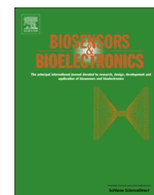

A simple and rapid chemiluminescence aptasensor for acetamiprid in contaminated samples: Sensitivity, selectivity and mechanism

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ABSTRACT

Ultralow concentration and selective detection of pesticide residue is important to evaluate the environmental and biological pollution and the threat to human health which single component pesticide can bring. Herein, we report an amplified chemiluminescence (CL) sensing platform for ultrasensitive and selective acetamiprid (widely used pesticide) detection. It is based on aptamer's high binding affinity to target and the relevance between AuNPs' morphology and its catalytic effect to stimulate the generation of CL in the presence of H_2O_2 and luminol. Moreover, AuNPs morphological slight change induced by aptamers' conformation during targets binding could lead to the significant change of catalytic properties. Therefore, the proposed sensing platform for pesticide residue exhibited a high sensitivity toward acetamiprid with a detection limit of 62 pM, which was about 100-fold lower than that of other aptamer-based sensor for acetamiprid detection. Because of the intrinsic specificity of aptamer's recognization, this sensing platform has high selectivity. So, this sensing platform provides a label-free and cost-effective approach for sensitive and selective detection of single component pesticide residue. More importantly, this CL method was successfully used to determine acetamiprid in real contaminated samples.

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1. Introduction

Pesticide residue is paid great attention in food safety and environmental pollution. As one of the most efficient insecticides, acetamiprid is widely used to control various insect pests. Because acetamiprid can cause colony collapse disorder, its residue released in wastewater and accumulated in soil and agricultural products will give rise to potential health risk of human beings, who are exposed to the contaminated environment (Ragas et al., 2011; Imamura et al., 2010; Qin et al., 2011). So, the monitoring and detection of acetamiprid have become very important. Current methods for acetamiprid detection were mainly the Association of Official Analytical Chemists methods including high performance liquid chromatography (Mohan et al., 2010; Obana et al., 2002), liquid chromatography (Watanabe et al., 2007), mass spectrometry (Park et al., 2011), gas chromatography (GC) (Zhang et al., 2008; Mateu-Sanchez et al., 2003), liquid chromatography-mass spectrometry and GC-mass spectrometry (Xie et al., 2011; Zhang et al., 2010; Radisic et al., 2009), and enzyme-linked immunosorbent assays (ELISA) (Watanabe et al., 2006). These methods require expensive instrument, complex sample preparation and purification procedures, and ELISA are susceptible to interferences. Therefore, it is highly desirable to develop a simple, fast and practicable method for the detection of acetamiprid in environment and agricultural products.

As an excellent molecular probe, aptamers are single-stranded oligonucleotides that are capable of binding all kinds of target ranging from metal ions, small molecules, macromolecules (such as proteins), even to viruses and cells with high affinity and specificity (Kawakami et al., 2000; Zuo et al., 2007; Bang et al., 2013; Wang et al., 2011, 2010), and are usually obtained through in vitro selection technique that is the systematic evolution of ligands by the exponential enrichment (SELEX) procedure (Bai et al., 2014; Alsager et al., 2014). Targets were recognized by that its binding could induce aptamers' conformational changes. Compared with other molecular recognition elements like antibody, aptamer possesses many merits such as simpler artificial synthesis and modification, easier storage, commercial availability, lower cost, and wider applicability. So, aptamer recognition has already been the important molecular recognition means and aptamer was also active in different sensing platform like fluorescence (Ozaki et al.,

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2006), chemiluminescence (CL) (Li et al., 2007, 2008; Zhang et al., 2009), electrochemiluminescence (ECL) (Fang et al., 2008), colorimetry (Yang et al., 2015) and electrochemistry (Schoukroun-Barnes et al., 2014; Li et al., 2014; Fan et al., 2013). Among them, CL detection has attracted an increasing interest due to its remarkable properties such as high sensitivity, wide calibration range, and simple instrumentation (Zeng et al., 2013; Dong et al., 2013). Recently, with the development of nanoscience, nanoparticles were introduced in the CL assay as catalysts to obtain high sensitivity. At the same time, aptamer-based CL sensing platforms involved nanoparticles have been focused in the last decade. Willner's group used the peroxidase-mimicking DNAzyme as an amplifying label for an aptamer and realized the CL determination of low-molecular weight substrates and proteins (Li et al., 2007). Similarly, Dong et al. developed an aptamer-based CL sensor for thrombin by using a peroxidase-mimicking DNAzyme as the catalytic label (Li et al., 2008). In 2009, Zhang et al. designed a CL aptamer-based system for adenosine triphosphate (ATP) detection in cancer cells based on CL resonance energy transfer (Zhang et al., 2009), and in their CL system, the aptamer was modified with an amino group to allow its attachment on the surface of magnetic nanoparticles. However, most of the developed CL sensors with nanoparticles and aptamer involved labeling and modifying procedure by either the catalytic amplifying label for an aptamer or modifying the aptamer with an amino group to allow the attachment. Such a labeling and modifying process makes the determination relatively more complicated, time consuming, and laborious (Li et al., 2009). More importantly, labeling or modifying can weaken the binding affinity between the target and their aptamers, so as to decrease the sensitivity (Ho et al., 2004). In addition, current aptamers selected for the pesticides is still limited and is not enough in comparison to the demand. Therefore, the application of aptamers as recognition elements in the nanoparticles-based CL sensors for pesticides residue detection was very few.

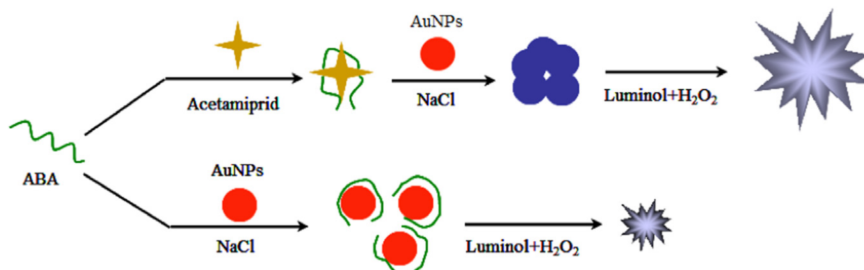
It's worth noting that the catalytic performance of gold nanoparticles (AuNPs) for CL reaction is closely related to its morphology. Our previous studies have found that the catalytic activity of aggregated AuNPs is stronger than that of dispersed AuNPs in the luminol–H_2O_2 CL system (Qi and Li, 2011, 2013; Qi et al., 2009, 2014a). Actually, AuNPs can effectively differentiate unstructured and folded aptamer by the following mechanisms (Wang et al., 2006). In the absence of target, the unfolded aptamer (ssDNA) can adsorb onto the surface of AuNPs and help to enhance the AuNPs' stability against salt-induced aggregation. In the presence of target, targets' binding makes aptamers' conformation to be changed from unfolded aptamer (ssDNA) to folded aptamer (e.g. hairpin structure). As for folded aptamer, the relatively rigid structure prevents the exposure of the DNA bases to the AuNPs and the high density of negative charges increases the repulsion between the DNA and the AuNPs (Wang et al., 2006). Thus the folded aptamer could not adsorb on the AuNPs and lose the ability to protect the AuNPs, and AuNPs aggregated. Based on this, a number of aptamer-based colorimetric sensors have been developed (Yang et al., 2015; Li et al., 2009; Wang et al., 2006; Shi et al., 2013). However, the sensitivity of colorimetric assay still encountered the challenge. It is well known that the demand of maximum residue limits (MRL) for pesticide around the globe becomes increasingly strict, which leads to the rapid development and wide use of high efficiency and low residual pesticide. Hence, it is extremely urgent for us to develop a rapid and high selective detection method with ultralow detection limit for extremely low concentration residual pesticide in environmental samples and agricultural products.

In this work, we design our aptamer-based CL sensors for acetamiprid determination by combining aptamer's molecular recognition with aggregated AuNPs' catalytic capability for luminol–H_2O_2 CL reaction. As shown in Scheme 1, by exploiting that targets-induced aptamers' conformational change lead to AuNPs' morphology change (from dispersed state to aggregated state), CL signal produced by the AuNPs could sensitively differentiate the conformational change of aptamer before and after adding the target acetamiprid. This assay strategy adequately embodies the specificity of aptamers' recognition and the sensitivity of AuNPs-based CL analysis, and the detection limit is estimated to be 62 pM (3σ), which is far more sensitive than aptamer-based colorimetric assay reported (Shi et al., 2013). The whole assay, label-free detection strategy for aptasensing, does not need aptamer labeling or modifying, and the assay process can be finished within 0.5–1 h. Thus, the problem in current acetamiprid determination, that is an urgent need to develop a rapid, sensitive and selective method, can be solved.

2. Experimental

2.1. Chemicals and apparatus

Acetamiprid was purchased from Aladdin Chemistry Co. Ltd. The 20-mer acetamiprid-binding aptamer (ABA) with the sequence of 5′-CTG ACA CCA TAT TAT GAA GA-3′ was adopted from the literature (He et al., 2011). ABA and control random sequence of 5′-AGA CAT GCC CAG ACA TGC CC-3′ were purchased from Shanghai Sangon Biotech Co., Ltd. (Shanghai, China). The ABA stock solutions (20 μM) were prepared in Tris–HCl buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.5) and diluted to the desired concentration with the same Tris–HCl buffer. About the preparation of aptasensor solution, each ABA was heated to 90 °C for 10 min, and slowly cooled to room temperature before use to unwind the single stranded oligonucleotide. ABA might be more unfolded if ABA cooled to 0 °C suddenly. Hence, the comparative experiments were carried out after ABA was heated to 90 °C for 10 min and cooled to 0 °C suddenly before use. The experimental result showed that there was no difference between the two preparations of aptasensor solution. In our aptasensor, all the experiments operational temperature was room temperature ($\approx$ 20 °C), which was also the optimal binding temperature for acetamiprid and ABA (Shi et al., 2013). Although ABA might be more unfolded when ABA cooled to



Scheme 1. Schematic illustration of the proposed CL assay for acetamiprid detection.

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