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Application of DNA aptamers as sensing layers for detection of carbofuran by electrogenerated chemiluminescence energy transfer

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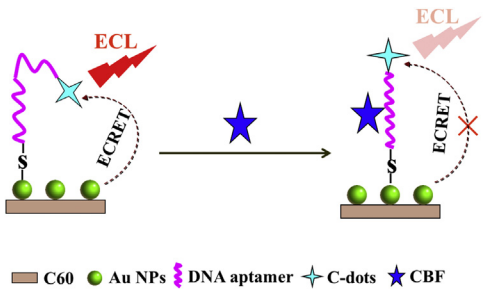
HIGHLIGHTS

- A new ECRET system between C60-Au and C-dots was first reported.
- C60-Au was introduced in the sensor to improve the selectivity of aptasensor.
- The aptasensor presents a generic detection strategy for detecting pesticide residues.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, an electrogenerated chemiluminescence (ECL) sensing platform for carbofuran detection was constructed based on ECL energy transfer (ECRET) and carbon dot (C-dot)-tagged aptamers as the recognition element. Fullerene (C60)-loaded gold nanoparticles (C60-Au) were used as the energy donor, modified on a glassy carbon electrode. C-dot-tagged DNA aptamers were used as the receptor, and ECRET then occurred between C60-Au and C-dots. After accepting the energy, the C-dots acted as a signal indicator and showed decreased signal intensity in the presence of targets, which competitively bound to DNA aptamers and blocked energy transfer. Using this robust, straight-forward strategy, the sensor showed a linear ECL response to carbofuran at concentrations from 2.0×10^{-11} mol L^{-1} to 8.0×10^{-9} mol L^{-1}. The detection limit of this assay was shown to be 8.8×10^{-13} mol L^{-1}. Thus, the sensing approach described in this study could be adapted for use in the detection of various pesticide residue targets.

1. Introduction

Carbofuran (CBF) [1] is a broad-spectrum carbamate pesticide that is commonly used in agriculture worldwide. CBF alone is highly toxic, and animal studies have suggested that CBF found in fresh produce may cause health problems. Therefore, it is necessary for us to detect and monitor CBF residues in farm products and the environment. Currently, CBF is mainly detected by high-performance liquid chromatography-mass spectrometry (HPLC-MS) [2], fluorescence [3], and electrochemical methods [4]. However, the use of HPLC-MS is limited owing to the high cost of operation and complex pretreatment requirements. Additionally,

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the stability and reproducibility of fluorescence and electronic methods need to be further improved, and CBF residues are present in trace quantities in real samples, i.e., less than 1×10^{-9} mol L^{-1}. Accordingly, it is necessary to develop a sensitive, selective, inexpensive, and simple method for the detection of CBF.

Aptamers [5,6], including DNA aptamers [7], RNA aptamers [8], and peptides [9], are oligonucleotide or peptide molecules that bind to specific target molecules. In recent years, aptamer-base sensors have attracted much attention in medical immunology [10], bioanalysis [11], and environmental analysis fields [12], due to the selectivity, affinity, versatile target binding, and relatively low cost of aptamers. Among these sensors, electrogenerated chemiluminescence (ECL) sensors [13] have the unique advantages of high sensitivity, wide linear range, low detection limit, convenience, and controllability. When the active substance (called the electrochemical donor) transfers energy to ECL reagents (acceptors) under a potential, the signal intensity of ECL is greatly enhanced; this phenomenon is referred to as ECL energy transfer (ECRET) [14,15]. Thus, the sensitivity of ECRET-based sensors is much higher than that of normal ECL sensors. However, no studies have reported the use of ECRET-based aptamer sensors for the detection of residual pesticides.

Fullerene C60 (C60) [16] has attracted much attention owing to its unique electron transport behaviour, optical and physical properties, mechanical features, and potential applications, including use as sensors [17], for gas storage [18], and as sensory reinforced metals [19]. C60 can combine with other inorganic [20,21] or organic materials [22] to form a composite material that exhibits excellent performance, possibly due to the synergistic effects of the added materials. C60 and its composites may function as energy and electronic donors [23]. Carbon dots (C-dots) [24], a new type of carbon nanomaterial, possess unique ECL properties [25], low toxicity [26], and good biocompatibility [27], making them promising materials for applications in ECL. Moreover, as novel ECRET reagents, C-dots have many excellent photoelectric properties that traditional organic molecules do not possess, including the dielectric effect, size distribution effect, and nano-confined effect for electronic and photonic molecules [28,29]. However, the application of ECRET as an ECL sensor using the C60 nano-composite and C-dots has not been studied.

In this study, we fabricated an ECL sensor based on ECRET between C60-loaded gold nanoparticles (C60-Au) and C-dots, with aptamers as recognition elements for CBF detection. C60-Au was synthesised using a simple one-step hydrothermal method and modified on a glassy carbon electrode (GCE). First, C-dots-tagged aptamer sequences were synthesized using systematic evolution of ligands by exponential enrichment (SELEX) [30]. Then C-dots-tagged aptamers were linked to the C60-Au-modified GCE by the thiol in the aptamers. Under certain conditions, ECL energy transfer occurred between C60-Au and C-dots, in which C60-Au acted as the energy donor and C-dots acted as the acceptor. After accepting the energy, the ECL of C-dots was greatly enhanced. In the process of CBF testing, C-dots acted as a signal indicator, with decreased signal intensity observed in the presence of the target after competitive binding of the target to DNA aptamers, blocking energy transfer (Fig. 1). The mechanism for the inhibition may be related to the tertiary structure of DNA aptamers. Before CBF binding to DNA aptamers, C-dots were most likely closer to the transduction surface, explaining the higher ECL output. Upon binding to the CBF, this structure was likely extended, leaving the C-dot farther away from the electrode surface and resulting in lower signals. The decrease in ECL intensity was linear with the CBF concentration, establishing a new method for CBF detection. Due to the ECRET and C60-Au amplification, the detection signals were amplified effectively. Therefore, the aptamer sensor exhibited high linearity, stability, sensitivity, and resolution and may have applications for the detection of CBF.

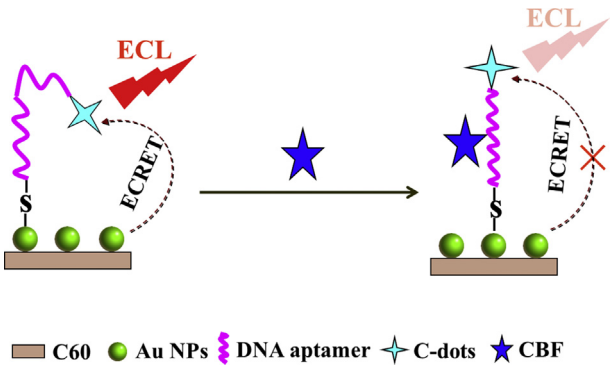

Fig. 1. Procedure for aptamer sensor fabrication for the detection of carbofuran (CBF).

2. Materials and methods

2.1. Chemicals, reagents, and electrochemistry

The ECL measurements were carried out on a Model MPI-E ECL analyser (Xi'an Remex Instrument Co. Ltd., China) using a three-electrode system. Electroanalytical measurements, including cyclic voltammetry (CV) and alternating current (AC), were performed on a standard three-electrode workstation (CHI660E; Shanghai Chenhua Instrument Co. Ltd., Shanghai, China). The three-electrode system consisted of an Ag/AgCl electrode containing saturated KCl solution as the reference electrode, a platinum wire electrode as the auxiliary electrode, and a modified gold electrode (d = 2 mm) as the working electrode.

CBF aptamer sequences were synthesised by Sangon Biotech Co. Ltd. (Shanghai, China), with the following sequence: 5′-C-dots–$(CH_2)_3$–CACCTGGGGGAGTATTGCGGAGGAAAGAGAACACTGG GGCAGATATGGGCCAGCAGGTC–$(CH_2)_3$–SH-3′, and stored at −20 °C out of direct light. CBF, aldicarb, imidacloprid, abamectin, dimethomorph, acetamiprid, carbary, metolcarb, pirimicarb, isoprocarb, fenobucarb, and tsumacide were obtained from the Agricultural Environmental Quality Supervision and Testing Centre of China. 6-Mercapto-1-hexanol (MCH) was obtained from Sigma-Aldrich and used without further purification. Additionally, 3×10^{-4} mol L^{-1} $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ solution was prepared with 0.5 mol L^{-1} KCl, and the molar ratios of $Fe(CN)_6^{3-}$:$Fe(CN)_6^{4-}$ was 1:1. All aqueous solutions were prepared using ultra-pure water (18.2 MΩ, Milli-Q, and Millipore).

2.2. Preparation of the C60-loaded gold nanoparticle-modified electrode

The GCE electrode was dipped into acetone and ethanol and then cleaned ultrasonically for 5 min. Next, C60-loaded gold nanoparticles were self-assembled on the GCE functionalised with 3-aminopropyltrimethoxysilane as previously reported [31]. GCE was prepared by treatment with toluene solution containing unmodified C60 (1.0×10^{-3} mol L^{-1}) and 4-aminothio-phenoxide/hexane-protected gold nanoparticles (1.5×10^{-4} mol L^{-1}). Then, the amination reaction of C60 facilitated self-assembly of nanoparticles on the GCE.

2.3. Fabrication of the aptasensor

The aptamer biosensor platform was constructed on a C60-Au-