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Simultaneous determination of zeatin and systemin by coupling graphene oxide-protected aptamers with catalytic recycling of DNase I

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ABSTRACT

Plant hormones are a collection of small molecules which play important roles in regulating the growth and development processes of plants. In this work, a sensitive and novel sensing platform for simultaneous multiplex detection of plant hormones is presented with choosing systemin and zeatin as the model targets. In the absence of the targets, the dye-labeled aptamers were adsorbed on the surface of graphene oxide (GO), result in the fluorescences of the dyes intensively quenched by GO which could also protect the aptamers from nuclease cleavage. While in the presence of the targets, the aptamers could be detached from the GO surface by specifically binding with targets. The fluorescences of the dyes were synchronously recovered and could be further amplified by DNase I catalytic recycling of self-produced reactants. So the two plant hormones can be simultaneously quantitatively determined by using synchronous scanning fluorescence spectrometry with no cross reaction between the two probes. The experimental results reveal that fluorescence intensities of two dyes exhibit good linear dependence on their target concentrations in the range of 0.67–6.0 μM and 0.33–5.0 μM with the detection limit of 60 nM and 35 nM for zeatin and systemin, respectively.

1. Introduction

Plant hormones are a structurally unrelated collection of small molecules that play a crucial role in regulating numerous aspects of plant growth, development, and response to a wide range of biotic and abiotic stresses [1]. On the basis of their diverse structures and physiological functions, plant hormones are categorized into several major classes including auxin (AUX), gibberellin (GA), abscisic acid (ABA), cytokinin (CK), salicylic acid (SA), ethylene (ET), jasmonate (JA), brassinosteroid (BR), nitric oxide (NO), strigolactone (SL), and polypeptide hormone, and it is likely that additional plant hormones are yet to be discovered [2–4]. Enormous studies had revealed the role of each class of hormone, but in recent years, molecular genetic studies have been elucidating complicated crosstalk among the plant hormones [5–7]. Therefore, the development of accurate identification and simultaneous quantitative determination of multiple classes of plant hormones would be very meaningful in plant researches and applications.

With the development of analytical techniques, more accurate and sensitive determination methods for plant hormones come into being, including immunoassays, electroanalysis, and especially chromatographic methods, such as high-performance liquid chromatography (HPLC), gas chromatography (GC), and capillary electrophoresis (CE) coupled with different detectors [8–11]. While these methods have unique advantages of high sensitivity, fast speed, efficient separation, and simultaneous identification of multiple plant hormones, they are limited to the expensive devices and complicated coupling operations.

Aptamers are short single-stranded oligonucleotides that selectively recognize a wide range of targets, from small organic/inorganic molecules to proteins [12–14]. Recently, panels of aptamers have been selected for two plant hormones, systemin [15] and zeatin [16]. Moreover, the aptamers have been applied to the detections of systemin and zeatin, respectively. Based on these works, aptamer-functionalized Zn^{2+} doped CdTe QDs have been synthesized and applied to systemin detection by our group [17]. However, the affinity between plant hormones and their aptamers are not strong enough, and each aptamer binds to only a single target molecule. These weak affinity and 1:1 binding ratio limits signal enhancement as well as the sensitivity of the assay.

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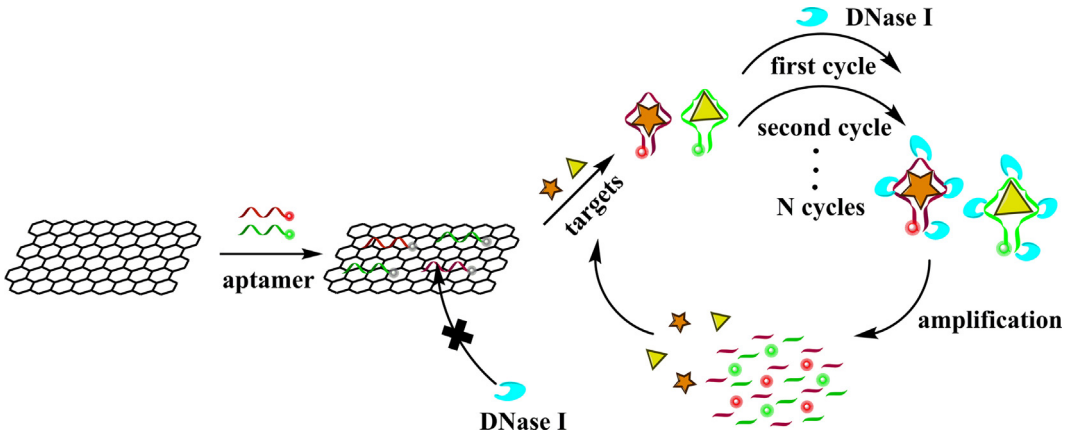

Fig. 1. The principle of cyclic enzymatic amplification method for simultaneous detection of zeatin and systemin based on GO protected and quenched aptamers.

To overcome these problems, combining GO-protected DNA probes and cyclic enzymatic amplification method (CEAM) [18], we developed a new platform for simple, sensitive and multiplex analysis of plant hormones. DNase I is an endonuclease that non-specifically cleaves DNA and releases di-, tri- and oligonucleotide products [19]. GO, a new kind of carbon nanomaterial, has been utilized for sensitive and selective detection of DNA [20,21] and proteins [22]. There are several advantages of GO-based fluorescent sensor [23–25]. First, GO has plenty of hydrophobic basal plane, which allow it to adsorb a wide range of biomolecules, such as Single-stranded DNA (ssDNA) on the surface via hydrophobic and p-stacking interactions [26,27]. Second, GO has been reported to be a super-quencher to various kinds of fluorophores, via fluorescence resonance energy transfer (FRET) or non-radiative dipole–dipole coupling. Third, GO can protect aptamers from nuclease cleavage due to the steric-hindrance effects [28–31]. Fourth, GO, as a nanocarrier, has ultra-high surface areas for loading, including multiple molecules for multiplex sensing. These characteristics of GO provide technological base for this novel sensing platform.

In this work, GO strongly bind aptamers and protect them from nuclease cleavage, accompanied with the quenching of fluorescence of fluorophores conjugated with aptamers. However, when binds with the plant hormone, the aptamer is in a stable state with the rigid structure and thus released from the substrate of GO. Furthermore, the free aptamer can be cleaved by nuclease, liberates the fluorophore and ultimately releases the plant hormone. Then, the released plant hormone binds to another aptamer on GO to initiate a next round of cleavage, which leads to significant amplification of the signal. Because of the cyclic use of plant hormone, theoretically one plant hormone can initiate numerous probe digestions, which leads to a highly sensitive detection of plant hormone. Since the wavelength intervals between the maximum excitation wavelength and maximum emission wavelength of FAM and ROX are very close, the fluorescence signals of two fluorophores can be obtained simultaneously by using synchronous scanning fluorescence spectrometry. Thus, the simultaneous detection of two plant hormones can be realized by measuring fluorescence intensities of FAM and ROX, respectively. Compared with the other GO-based fluorescent sensor, the emergence of nuclease cleavage provides exciting new possibilities for achieving high sensitivity. What is more, the GO-protected multiplexed sensing strategy is firstly applied in the detection of plant hormones as we know.

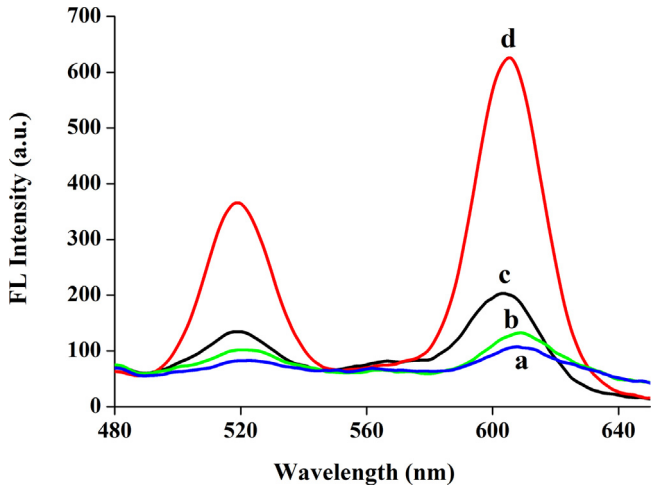

Fig. 2. Synchronous scanning fluorescence spectrum for the specificity analysis with (a): GO, Zeatin probe and Systemin probe; (b) GO, Zeatin probe, Systemin probe, zeatin and systemin; (c): GO, Zeatin probe, Systemin probe and DNase I; (d): GO, Zeatin probe, Systemin probe, zeatin, systemin and DNase I. Experimental conditions: GO, 5 μg mL^{-1}; Systemin probe, 5.0 nM; Zeatin probe, 4.0 nM; systemin, 5.0 μM; zeatin, 5.0 μM; DNase I, 0.5 U.

2. Materials and methods

2.1. Apparatus and chemicals

DNA sequences were synthesized by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China) and shown in the following:

1) Systemin probe: 5′-ROX-CTAACGGGTTTCGGGGGG GTAGGGAGGTTAG-3′;
2) Zeatin probe: 5′-FAM-CGGATATGGTTAGGCAGGCATAAGAGGT TTATCCG-3′.

Peptide sequences were synthesized by ChinaPeptides Co., Ltd. (Shanghai, China) and shown in the following:

1) Systemin: Ala-Val-Gln-Ser-Lys-Pro-Pro-Ser-Lys-Arg-Asp-Pro-Pro-Lys-Met-Gln-Thr-Asp;
2) Peptide 1: Cys-Cys-Val-Trp-Thr-Cys-Asp-Lys-Gly;
3) Peptide 2: Val-Val-Val-Thr.

All DNA samples were dissolved in 10 mM Tris–HCl buffer solution (pH 7.4, 150 mM NaCl, 10 mM $MgCl_2$, 10 mM KCl) and stored at 4 °C for use.

DNase I was obtained from Takara Biotechnology (Dalian) Co., Ltd. (China). GO was purchased from Sinocarbon Materials Technol-