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Research Paper

Photoelectrochemical apta-biosensor for zeatin detection based on graphene quantum dots improved photoactivity of graphite-like carbon nitride and streptavidin induced signal inhibition

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ABSTRACT

Zeatin is a kind of typical cytokinin, which plays important roles in regulating the proliferation and differentiation of plant cells, and widely distributed in various plant tissues. Herein, a simple and sensitive photoelectrochemical method was developed for zeatin detection using graphite-like carbon nitride (g-C_3N_4) as photoactive material, graphene quantum dots as photoactivity improvement reagent, gold nanoparticles as immobilization substrate for DNA probe, DNA biotin labeled aptamer as zeatin recognition and streptavidin capture element, and streptavidin as PEC response inhibitor factor. In the presence of zeatin, DNA aptamer can bind with zeatin to form conjugate, which make aptamer releasing from electrode surface and decrease the capture amount of streptavidin, causing the increase of the PEC response. Under optimal conditions, the biosensor showed wide linear range from 0.1 to 100 nM and low detection limit of 0.031 nM (3σ). The developed method also presented good selectivity, even discriminating zeatin analogues. In addition, the proposed detection strategy can be further applied to detect zeatin in complex biological matrix, revealing great potential in practical applicability. This work might provide an excellent platform for zeatin or other phytohormone detection using aptamer as recognition reagent.

1. Introduction

Cytokinins (CKs), a family of N_6-substituted adenine derivatives, are a kind of crucially natural phytohormone, which can regulating the proliferation and differentiation of plant cells, and also control diverse processes in plant growth and development, including seed germination, de-etiolation, chloroplast differentiation, apical dominance, plantpathogen interactions, flower and fruit development, and leaf senescence [1,2]. Zeatin is the first identified naturally occurring cytokinin extracting from the grain filling period of sweet corn, which is the major active cytokinin and widely distributed in various plant tissues [3,4].

With the fast development of analytical techniques, more sensitive and accurate detection methods for zeatin have been achieved, including mass spectrometric technique sans chromatographic separation [5], gas chromatography-mass spectrometry [6], high-performance liquid chromatography with electrospray ionization tandem mass spectrometry [7], liquid chromatography-mass spectrometry [8]. Though these methods have the advantages of high sensitivity and simultaneous detection of multiple phytohormones, they still require expensive instruments, skill operators, complicated pretreatment process, which limit their widely application for *in situ* assay.

Recently, photoelectrochemical (PEC) biosensor is a newly and promising determination technique based on the electron transfer among analyte, photoactive species and electrode under photo-irradiation. Due to the advantages of low background current, high sensitivity, fast response, low cost, and easy miniaturization of detection devices [9–11], PEC biosensor has been widely applied for detecting various analytes, such as DNA [12,13], microRNA [14,15], protein [16,17], small organic molecule [18,19], and metal ion [20–22]. For PEC analysis, a photoactive working electrode is required to provide photocurrent under photo-irradiation. Recently, graphite-like carbon nitride (g-C_3N_4) has attracted great attentions due to its high photocatalytic efficiency. As a kind of semiconductor material, g-C_3N_4 nanosheet also possesses photoelectric conversion performance and can produce photocurrent under visible light irradiation [23]. However, g-C_3N_4 nanosheets exist in the disadvantage of low photoelectric conversion efficiency. To improving this deficiency, composite g-C_3N_4 semiconducting

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materials with enhanced photo-activity, such as nitrogen-doped graphene/layered MoS_2 [24], TiO_2 [25], CdS quantum dots [16], reduced graphene oxide [26], have been adopted to substitute single semiconductor, which has been supposed to promising strategy for improving photoactivity of g-C_3N_4. These modifications probably could improve the detection sensitivity of PEC biosensor.

To achieve high detection specificity, specific recognition reagent for zeatin is also needed for PEC biosensor construction. Previously, antibody has been widely employed for specific recognition of zeatin [27]. However, the activity of antibody can be influenced greatly by the storage conditions and experimental conditions, and the antibody is also expensive. Aptamers are a kind of single-strand DNA or RNA with high affinity to its target. Compared with antibody, aptamers possess advantages of high affinity, low cost, easy synthesis and modification, remarkable target diversity and good stability [28,29]. On account of these benefits, aptamers have been widely applied in biosensor fabrication and various targets detection, such as metal ions [30,31], small molecule contaminants [32,33], proteins [34,35], bacteria [36,37] and viruses [38–40]. More importantly, zeatin aptamer has also been screened and two fluorescence methods based on aptamer for zeatin detection was developed [41,42]. However, no electrochemical-based method was investigated.

In this work, g-C_3N_4 nanosheets and graphene quantum dots (GQDs) was explored for developing a PEC biosensing platform for the first time, where g-C_3N_4 nanosheets was served as highly efficient photoactive material, biotin-labeled zeatin-binding aptamer was employed as zeatin recognition element, and streptavidin was used as signal inhibition reagent. After composited with GQDs, the PEC response of g-C_3N_4 nanosheets improved greatly due to the excellent synergy effect between the PEC activities of g-C_3N_4 and GQDs, reducing the probability of the recombination of photogenerated electron and hole. In the presence of zeatin, the aptamer will bind with zeatin, and release from the double-strand DNA (dsDNA) on electrode surface, which make the failure of the formation of biotin-streptavidin conjugate on the electrode surface, and leads to a strong photocurrent. Based on it, a sensitive and selective PEC apta-biosensor was constructed for zeatin detection.

2. Experimental section

2.1. Reagents and instruments

Melamine, ascorbic acid (AA), *trans*-zeatin sodium citrate, chloroauric acid ($HAuCl_4$), dithiothreitol (DTT), tris(2-carboxyethyl)phosphine (TECP), 6-benzylaminopurine, gibberellin, indole-3-acetic acid, indole-3-propionic acid, indole-3- butyric acid were purchased from Aladdin (Shanghai, China). Mercaptopropionic acid (MPA) was supplied by Sigma-Aldrich (USA). Tris-base was obtained from Solarbio (Shanghai, China). Streptavidin (SA), exonuclease I (Exo I) and DNA were purchased from Sangon (Shanghai, China), and DNA sequences were listed as follows. Probe DNA, 5′-SH-CGG ATA AAC CTC TTA TGC CTG CCT AAC CAT ATC CG-3′, aptamer DNA, 5′-biotin-CGG ATA TGG TTA GGC AGG CAT AAG AGG TTT ATC CG-3′. Exo I storage buffer, 20 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM DTT and 50% (v/v) glycerol. Exo I reaction buffer, 67 mM glycine-KOH (pH 9.5), 6.7 mM $MgCl_2$, 1 mM DTT. DNA dissolution buffer (1×TE buffer), 10 mM Tris–HCl, 1 mM EDTA, pH 8.0. DNA immobilization buffer, 10 mM Tris-HCl, 1.0 mM EDTA, 1.0 M NaCl, and 1.0 mM TCEP, pH 7.0. DNA hybridization buffer, 100 mM NaCl, 20 mM Tris–HCl, 2 mM $MgCl_2$, 5 mM KCl, pH 7.5. Detection buffer, 10 mM PBS containing 0.1 M AA, pH 7.4.

Transmission electron microscopy (TEM) images were obtained from JEM-1400PLUS (Japan).

2.2. Preparation of g-C_3N_4 nanosheets

2.0 g melamine was loosely packed in a porcelain boat, and then heated in a Muffle furnace with heating rate of 20 °C/min to 550 °C for 4 h [43]. The obtained yellow product was washed with ethanol and redistilled deionized water for three times, respectively. Then, the product was dried at 60 °C for 6 h. Afterwards, 2 mg g-C_3N_4 was weighed accurately and dispersed in 4 mL redistilled deionized water with the aid of ultrasonication for 30 min. Subsequently, g-C_3N_4 suspension liquid was treated with probe sonicator (Scientz® JY92-IIDN, Zhejiang, China) for 30 min with 300 cycles of 3 s of sonication with an interval of 3 s. Then, the suspension liquid was centrifuged at 10,000 rpm for 30 min. The solid product was collected and washed with redistilled deionized water for three times. Finally, the product was dried at 60 °C for 6 h.

2.3. Synthesis of gold nanoparticles (AuNPs)

AuNPs were synthesized according to previous report [44]. In brief, 200 mL two-neck flask, magnetic stir bar, glass stopper and spherical condenser in aqua regia for at least 15 min. Then the glassware was rinsed with redistilled deionized water. After that, 98 mL of redistilled deionized water and 2 mL of 50 mM $HAuCl_4$ were added into the two-neck flask. Afterwards, the mixed solution was heated to reflux. Then, 10 mL of 38.8 mM was added into the flask, and the reaction system was allowed to reflux for another 20 min. Subsequently, the solution was naturally cooled to room temperature under stirring. Finally, the deep red solution was obtained.

2.4. Biosensor construction

The ITO electrode was first cleaned in acetone, ethanol/NaOH (1:1, volume ratio, NaOH concentration is 0.1 M) and deionized water each for 30 min ultrasonically. Then, 40 μL of 2.0 mg/mL g-C_3N_4 dispersion was dripped on ITO electrode surface, and dried under the irradiation of infrared lamp. The electrode was noted as g-C_3N_4/ITO. After that, g-C_3N_4/ITO electrode was further coated with 40 μL of 0.25 mg/mL GQDs and 40 μL AuNPs dispersion successively. The obtained electrode was labeled as GQDs/g-C_3N_4/ITO and, respectively. Then, AuNPs/GQDs/g-C_3N_4/ITO was incubated with 20 μL of 1.0 μM probe DNA for 120 min at room temperature in a humid cell. The electrode was marked as ssDNA/AuNPs/GQDs/g-C_3N_4/ITO. The electrode was then sealed with 20 μL of 0.1 mM MPA for 60 min. DNA hybridization was performed by incubating ssDNA/AuNPs/GQDs/g-C_3N_4/ITO with 20 μL of 1 μM aptamer DNA for 120 min at 37 °C under humid conditions. The electrode was named as dsDNA/AuNPs/GQDs/g-C_3N_4/ITO. Afterwards, the electrode was incubated with 20 μL of Exo I buffer containing 100 unit/mL Exo I for 60 min at 37 °C, and followed with 20 μL of 0.1 mg/mL streptavidin (in 10 mM Tris-HCl and 50 mM NaCl) for 75 min. The electrode was noted as SA/dsDNA/AuNPs/GQDs/g-C_3N_4/ITO.

For zeatin detection assay, 20 μL of different concentrations of zeatin (in 10 mM Tris-HCl and 50 mM NaCl, pH 7.4) was dripped on dsDNA/AuNPs/GQDs/g-C_3N_4/ITO surface and incubated at room temperature for 120 min. Then, the electrode was further incubated with Exo I and streptavidin successively.

After each modification process, the electrode was rinsed with washing buffer for 3 mins, and dried under N_2 blowing.

2.5. PEC detection

PEC detections were performed at a homemade PEC system. A 500 W Xe lamp was served as the irradiation source to generate the visible-light with the assistance of optical filter. The photocurrent was obtained at CHI832A electrochemical workstation (CHI instru-