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DNA-based hybridization chain reaction for signal amplification and ultrasensitive chemiluminescence detection of gibberellic acid

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ABSTRACT

A novel chemiluminescent (CL) immunoassay protocol for the sensitive determination of Gibberellic acid (GA) using DNA-based hybridization chain reaction (HCR) is described. The carboxyl terminated magnetic beads (MBs) were modified with GA antibody and the gold nanoparticles (AuNPs) signal probes were labeled with the GA antibody and DNA initiator strands. In the presence of target GA, the sandwiched immunocomplex was formed between the immobilized antibody on the MBs and the signal antibody on the AuNPs. When the two complementary stable species of DNA hairpins, which are G-rich DNA, were added the hybridization events were happened, thereby resulting forming the long nicked double-helix. Numerous double G-rich DNA were formed on the AuNPs, each of which produced a CL signal within the applied CL reaction. The CL signal was obtained via the instantaneous derivatization reaction between a specific CL reagent, 3,4,5-trimethoxyl-phenylglyoxal (TMPG), and the G-rich DNA on immunocomplex. Under optimal conditions, GA can be detected in the concentration ranged from 0.01 ng/mL to 70 ng/mL, and the limit of detection was 0.003 ng/mL. The reproducibility and selectivity of the developed method were also investigated. In addition, the real samples were assayed by using the developed immunoassay, and the recovery was 96.0–102.0%.

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1. Introduction

Gibberellic acid (GA, (2,4α,7-trihydroxy-1-methyl-8-methylenegib-3-ene-1,10-dicarboxylic acid 1,4α-lactone)) is a diterpenoid compound in a class of gibberellins. GA is a plant hormone that promotes seed germination, stem elongation, premature flowering, and cone production and retards leaves and fruit senescence [1]. Although low mammalian and environmental toxicity plant hormone, their extensive use may be cause harm. For example, in 2011, the incident of exploding watermelon caused a very adverse impact on the watermelon industry because of the abuse of forchlorfenuron. The residue level of plant hormone in foods, especially in fruits, received more and more attention [2].

Traditional techniques used for determination of GA and other plant hormones include spectrophotometry [3], high performance thin layer liquid chromatography [4], gas chromatography [5,6], self-assembling of phosphotungstic acid-graphene oxide nanohybrid on graphite electrode-based electrochemical sensor [7], high-performance liquid chromatography (HPLC) [8–11], synchronous derivative spectrofluorometry [12] and immunoassays [13].

Recently, some new methods such as potentiometric detection [14,15], micellar electrokinetic chromatography [16], photochemically induced fluorescence [17] and liquid chromatography–tandem mass spectrometry [2,18–21], capillary electrophoresis with laser-induced fluorescence detection [22] were developed. But these methods are laborious, expensive or lower sensitivity.

In recent years, signal amplification strategies by using DNA as amplified indicators have attracted great attention. The DNA based amplification strategies mainly included polymerase chain reaction (PCR), ligase chain reaction (LCR), rolling circle amplification (RCA) and hybridization chain reaction (HCR) [23,24]. Among these methods HCR is non-enzymatic signal amplification strategy. The HCR technique, based on a chain reaction of recognition and hybridization events between two sets of DNA hairpin molecules, offers an enzyme-free alternative for rapid detection.

The most advantage of HCR over other amplification methods is that it allows for selective and specific extension at room

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temperature without enzymes. And many method based on HCR have be reported. For example, Choi et al. used HCR to enhance the sensitivity and accuracy of signals detected from sandwich immunoassays for analytes captured from single viable cells [25]. Niu et al. designed a DNA nanomachine system based on HCR and combined with enzyme-amplification to detect DNA [26]. Huang et al. combined the amplification capability of HCR with the spatially sensitive fluorescence signal of pyrene molecules to detect nucleic acids in complex biological fluids [27]. Song et al. reported a novel assay for the detection of platelet-derived growth factor BB via HCR based on an aptameric system [28]. Zhang et al. developed a sandwich type electrochemical immunoassay protocol for determination of human IgG by using gold nanoparticles and DNA-based HCR technique [29]. And the most reported methods based HCR are kinds of labeled methods. They need two labeled hairpin probes. Such labeling can increase the steric hindrance or change the conformation of hairpin probes and thus might decrease the target–probe hybridization efficiency. And the complicated hairpin probe modification process with indicators is unsuitable for routine use [30]. To solute these problems, the label-free hairpin probes for the HCR assembly to achieve promising results for the detection of target in the immuno-HCR assay were also developed [31,32].

This article reports the determination of gibberellic acid (GA) by a new instantaneous derivatization reaction chemiluminescent (CL) immunoassay protocol using the antibody and G-rich DNA labeled AuNPs as CL probe coupling HCR with instantaneous derivatization technology. In the presence of target GA, the sandwiched immunocomplex could be formed between a GA antibody-modified MB and GA antibody and DNA1 labeled AuNPs. Following that, H1 and H2 were added. The carried initiator strand (DNA1) on AuNPs initiates the HCR between H1 and H2, resulting in the formation of double DNA on MB. After that, CL detection was performed through an instantaneous derivatization reaction between 3,4,5-trimethoxyl-phenylglyoxal (TMPG) and G bases within the formed double DNA on MB. This method offered a new enzyme-free and label-free hairpin probes analytical technology. It held great promise for protein diagnostics, microarrays, and microchips, as well as for bioanalysis in general.

2. Experimental

2.1. Reagent and instruments

3,4,5-Trimethxylphenylglyoxal (TMPG) kindly was donated by Prof. Jianzhong Lu and Masaaki Kai (Prof. Lu: School of Pharmacy, Fudan University, China; Prof. Kai: School of Pharmaceutical Sciences, Nagasaki University, Japan). Tri(2-carboxyethyl)phosphine hydrochloride (TCEP, 98%), indole-3-acetic acid (IAA), Gibberellic acid (GA), Kinetin (Kt) and abscisic acid (ABA) were purchased from Alfa Aesar (Massachusetts, USA). GA antibody was purchased from Biocompare (CA, USA), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS) and bovine serum albumin (BSA) were purchased from Sigma (St. Louis, USA). Carboxyl groups modified magnetic beads (Carboxyl modified MBs, 0.5 μm, 10 mg mL^{-1}) were purchased from Baseline Chromtech Research Centre (Tianjin, China). And other chemical reagents were bought from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All chemicals used were of analytical grade and were used as received. All solutions were prepared with deionized and autoclaved water from a Millipore system.

0.1 M phosphate buffer (pH 7.4) was prepared by dissolving 0.13 g KH_2PO_4, 1.9 g $Na_2HPO_4{\cdot}12H_2O$ in one liter water. 0.15 mol l^{-1} PBS buffer, pH 7.4 was prepared by dissolving 0.2 g KH_2PO_4, 2.9 g $Na_2HPO_4{\cdot}12H_2O$, 8.0 g NaCl and 0.2 g KCl in one liter water. PBST solution was prepared by PBS solution containing 0.05% (v/v) Tween-20.

Oligonucleotides were obtained from SBS Genetech Co. Ltd. (Beijing, China). Their sequences were presented in following:

DNA1, 5′-SH-CCCCAACTCCTCCCA$(A)_{10}$CAAAGTAGTCGAGGCCC-3′ (thioled on AuNPs)

H1, 5′-AGTCGAGGCCCCGGCGTGGGTTAACACGCCGGGGCCTC-GACTACT TTG-3′;

H2, 5′-TTAACCCACGCCGGGGCCTCGACTCAAAGCCTAGTCGA-GGCCCC GGCGTG -3′.

CL emission was detected with a IFFS-E multifunction CL analyzer (Remex Analytical Instrument Co. Ltd., Xi'an, China). A TGL-16G centrifuge (Shanghai Anting Science Instrument Co., China) was used for centrifugation.

2.2. Preparation of antibody labeled MBs

The antibody was immobilized on the MBs according to the protocol provided by Zhang [33]. 200 μL of 10 mg mL^{-1} carboxylated MBs was added into a 2 mL Eppendorf tube. The MBs were washed three times with 400 μL of 0.1 M imidazol–HCl buffer (pH 7.0) and then resuspended to a final volume of 400 μL in the same buffer solution. Then 400 μL of 0.25 M NHS and 0.5 M EDC solution was added to the Eppendorf tube and the mixture was incubated at 37 °C for 30 min, followed by washing three times with 400 μL of 0.1 M phosphate buffer (pH 7.4) and resuspending to a final volume of 400 μL. Then the resulting antibody labeled MBs was stored at 4 °C for further use.

2.3. Preparation of antibody and DNA1 labeled AuNPs

Antibody and DNA1 labeled AuNPs were prepared according to the literature with a little modification [34,35]. 200 μL of polyclonal goat anti-human IgG antibody (2 mg mL^{-1}) was added into 1.0 mL of 20 nm AuNPs solution and incubated for 30 min at room temperature. Then the 100 μL of 10^{-6} M DNA1 which was activated with TCEP (10 mM) was added. After shaking gently for 16 h at room temperature, the antibody and DNA1 labeled AuNPs were aged in the solution (0.3 M NaCl, 10 mM Tris-acetate, pH 8.2) for another 24 h. The resulting antibody and DNA1 labeled AuNPs were washed with 2.0 mL of 0.1 M phosphate buffer (pH 7.4) for three times, and resuspended in 2.0 mL PBS and stored at 4 °C for further use.

2.4. Analytical protocol

The developed CL assay is illustrated in Scheme 1. The chemiluminescent assay involved adding 10 μL the antibody labeled MBs into 1.5 mL centrifuge vials containing 20 μL target. The immunological reaction proceeded at room temperature with gentle mixing for 30 min. After the magnetic separation, the MBs coated with the antibody–antigen were washed twice with 50 μL PBST buffer and suspended in 40 μL PBS buffer. Then 20 μL antibody and DNA1 labeled AuNPs was added and mixed with gentle shaking at room temperature. After incubation for 30 min, a magnetic separation and multiple washing with PBST buffer (50 μL) were then carried out. Then 20 μL mixture solution containing 0.5 μM H1 and 0.5 μM H2 was added. After incubation for 70 min at RT, the MBs were washed three times with 50 μL PBST buffer and suspended in 40 μL PBS buffer. And then the resulting solution was taken for CL detection.

2.5. CL measurements

The CL detection was carried out with an IFFS-E multifunction CL analyzer. Firstly, a 2 mL tube was placed in the luminescence

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