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Imaging of jasmonic acid binding sites in tissue

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ABSTRACT

Hormones regulate the mechanism of plant growth and development, senescence, and plants' adaptation to the environment; studies of the molecular mechanisms of plant hormone action are necessary for the understanding of these complex phenomena. However, there is no measurable signal for the hormone signal transduction process. We synthesized and applied a quantum dot-based fluorescent probe for the labeling of jasmonic acid (JA) binding sites in plants. This labeling probe was obtained by coupling mercaptoethylamine-modified CdTe quantum dots with JA using *N*-hydroxysuccinimide (NHS) as a coupling agent. The probe, CdTe–JA, was characterized by transmission electron microscopy, dynamic light scattering, and fluorescent spectrum and applied in labeling JA binding sites in tissue sections of mung bean seedlings and *Arabidopsis thaliana* root tips. Laser scanning confocal microscopy (LSCM) revealed that the probe selectively labeled JA receptor. The competition assays demonstrated that the CdTe–JA probe retained the original bioactivity of JA. An LSCM three-dimensional reconstruction experiment demonstrated excellent photostability of the probe.

Plant hormones regulate numerous processes in plant growth and development, are involved in stress response, and have different effects on plant germination, root growth, reproduction, aging, and resistance to pests and diseases [1,2]. Plant hormones have been recognized to be of great importance in improving the yield and quality of agricultural products. However, the molecular mechanisms of plant hormone actions are not very clear. The general mechanism involves the perception of certain cellular sites by a plant hormone, following which physiological and biochemical reactions are activated after the hormone binds selectively to receptors. Binding sites are the first components in the hormone and plant interaction process. Therefore, it is necessary to detect plant hormone binding sites in order to understand the molecular mechanisms behind hormone action. The detection of plant hormone binding sites and their distribution will comprise the theoretical basis for identifying specific binding proteins and cloning receptor genes.

However, most hormones and receptors have no obvious photoelectric signal. The detection of binding sites is not feasible using readily available and convenient optical methods. The lack of applicable methods hinders research in this area. If a newly developed fluorescent probe retaining the original biological activity of the hormone is introduced and the fluorescence signal of the probe can be easily measured, the plant hormone binding sites and distribution can be detected via fluorescence imaging methods. For prolonged, dynamic, real-time in situ observation, this fluorescent probe should be able to avoid the photobleaching phenomenon observed when using traditional organic dye probes.

Jasmonic acid (JA)[1] is an important plant hormone and can improve plant defenses against pathogenic microorganisms and pests. The evidence from molecular biology experiments demonstrates that JA is indispensable in plant defense responses and many other aspects of plant growth and development [3,4]. JA has become a major focus for plant hormone research during recent years [5–7]. However, there are very few reports on JA binding protein research. Yan and coworkers [8] built a high-quality structural model of COI1, a jasmonate receptor, and performed molecular modeling of COI1–jasmonate interactions. They demonstrated that COI1 is a receptor for jasmonate using the immobilized jasmonate, surface plasmon resonance technology, and photoaffinity labeling technology. They also described the molecular interactions between COI1 and jasmonate. JA–Ile binds to COI1 mainly via the keto group of the cyclopentanone ring, the pentenyl side chain, and the oxygen atom of the amide group of JA–Ile. Gu and coworkers [9] designed and synthesized three biotin-tagged photoaffinity probes for JAs to determine whether COI1 binds directly to jasmonates and compared the structure and biological activity of these probes. However, the

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[1] *Abbreviations used:* JA, jasmonic acid; QD, quantum dot; CaM, calmodulin; LSCM, laser scanning confocal microscopy; CdTe–JA, JA-conjugated QD probe; MA, mercaptoethylamine; NHS, *N*-hydroxysuccinimide; 3D, three-dimensional; MJA, methyl jasmonate; DLS, dynamic light scattering; TEM, transmission electron microscopy; IR, infrared; UV–Vis, ultraviolet–visible; PBS, phosphate-buffered saline; EDC, 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride.

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organic photoaffinity probes do not have a fluorescence imaging function. Ueda and coworkers [10] conjugated aglycone JA with fluorescein isothiocyanate (FITC) to obtain a fluorescence-labeled probe and studied the specific receptor in the leaves of *Albizia* plants. However, there are no reports on visualization research on JA binding sites and distribution or on JA binding proteins. In this study, JA was selected as a hormone template to evaluate and validate the detection method for the binding sites.

The application of quantum dots (QDs) in cytology is expanding [11–14] but has rarely been used in plant systems. To identify the receptor sites of calmodulin (CaM), Wang and coworkers [15] prepared a CaM-conjugated QD system for single-molecule-level detection at the surface of plant cells. This approach showed that QD–CaM binds selectively to sites on the outer surface of the plasma membrane using laser scanning confocal microscopy (LSCM) and other methods.

To address the problem of the lack of a measurable signal from JA binding to special proteins, we employed the JA-conjugated QD (CdTe–JA) probe for labeling JA binding sites in plant tissue sections and roots. In this study, CdTe–JA was obtained for the first time through coupling mercaptoethylamine (MA)-modified CdTe QDs with JA using *N*-hydroxysuccinimide (NHS) as a coupling agent. We used CdTe–JA as a targeted optical probe for fluorescence imaging of the root tissue sections of mung bean seedlings and *Arabidopsis thaliana*, as outlined in Scheme 1. LSCM results showed that the probe could selectively label JA binding sites. Competition assays revealed that the CdTe–JA probe retained the original biological activity of JA. LSCM results of a three-dimensional (3D) reconstruction experiment demonstrated that the probe had excellent photostability.

Materials and methods

Materials

All chemicals used were of analytical grade or of the highest purity available. All solutions were prepared with double distilled water. Mercaptoethylamine hydrochloride (MA·HCl, 99%), methyl jasmonate (MJA, 95%), tellurium powder (99.9%), and $NaBH_4$ (99%) were purchased from Aldrich Chemical. $CdCl_2{\cdot}2.5H_2O$ (99.9%) was purchased from Shanghai Reagent (China). NHS was obtained from J&K (China). Isopropanol was purchased from Guangzhou Chemical Reagent (China).

Instrumentation

The nanoparticle diameters and nano-collosol zeta potential were determined by dynamic light scattering (DLS) with a Zetasizer Nano ZS nanoparticle size analyzer (Malvern Instruments, UK). Confocal fluorescence imaging was performed using an LSM 510 Meta Duo Scan laser scanning confocal microscope (Carl Zeiss, Germany) with 488 nm laser excitation. Fluorescence imaging was accomplished using a Nikon Eclipse 50i fluorescence microscope (Nikon, Japan). Transmission electron microscopy (TEM) images were obtained using a JEM-2100 HR microscope (JEOL, Japan). Fluorescence spectra were obtained with an F-2500 spectrofluorometer (Hitachi, Japan). The infrared (IR) spectra were recorded using a Prestige-21 FT-IR spectrometer (Shimadzu, Japan). Ultraviolet–visible (UV–Vis) absorption spectra were measured with a UV–Vis 1700 spectrophotometer (Techcomp, China).

Experimental methods

Synthesis and purification of CdTe–JA conjugate

JA was obtained from alkaline hydrolysis of MJA [16]. CdTe QDs were synthesized in aqueous solution with MA as the stabilizer following the previously published procedures [17]. Briefly, CdTe QDs were synthesized in aqueous solution by reaction of $CdCl_2{\cdot}2.5H_2O$ and NaHTe in the presence of MA, refluxing at 100 °C.

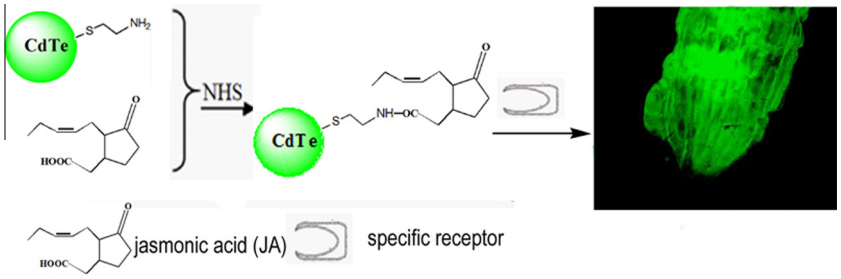
A sample of 0.0673 g of JA was dissolved in 1.0 ml of dimethylformamide (DMF) and then diluted to 10.0 ml with double distilled water. An aliquot (320 μl) of 0.1 mol/L NHS was mixed with 1.0 ml of JA solution (3.2×10^{-5} mol). The mixture was activated at 37 °C for 5 min [18], and then 5.0 ml of purified CdTe QDs solution was added under uniform stirring (the molarity according to MA count was 9.6×10^{-5} mol, and the molar ratio of MA/NHS/JA was 3:1:1). The resulting mixture was stirred for 30 min at 37 °C in the dark and refrigerated at 4 °C overnight to stop the reaction. The concentration of CdTe–JA solution was 2.0×10^{-4} M according the dosage of HTe^-. The CdTe–JA solution was dialyzed at 4 °C in the refrigerator by using dialysis bags (MWCO = 14,000 Da) and stored at 4 °C in the dark after being dialyzed for approximately 2.5 h.

Selectivity labeling of JA binding sites with the CdTe–JA probe in plant tissue sections

The root sections of mung bean seedling or *A. thaliana* root tips were placed in centrifuge tubes and incubated with double distilled water, CdTe, and CdTe–JA conjugates in a darkroom for 2 h at 25 °C and then washed with phosphate-buffered saline (PBS, pH 7.4) three times. The root sections and root tips were then examined under a confocal fluorescence microscope. The samples were viewed with a 40× oil immersion lens objective [19], the 488-nm argon laser was used, and emission in a 530- to 600-nm wavelength range was recorded. Post-acquisition image handling was done with Zeiss AIM software.

Assays of competitive combination between CdTe–JA and JA to JA binding sites in tissue sections

A series of competition experiments were carried out at 25 °C according to previous methods with some modifications [15]. Mung bean seedling root sections were first incubated for 1 h in 2 ml of 0 to 5.0×10^{-2} mol/L JA solution, taken out, and then transferred to 2 ml of 1.0×10^{-4} mol/L CdTe–JA solution. After 1 h of incubation, the sections were washed three times with PBS (pH 7.4) and fluorescence images were obtained by a fluorescence



Scheme 1. CdTe–JA probe synthesis and combination with the JA receptor. MA-capped CdTe QDs were activated by NHS and coupled with JA to form the CdTe–JA probe.