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Visualization of hormone binding proteins in vivo based on Mn-doped CdTe QDs

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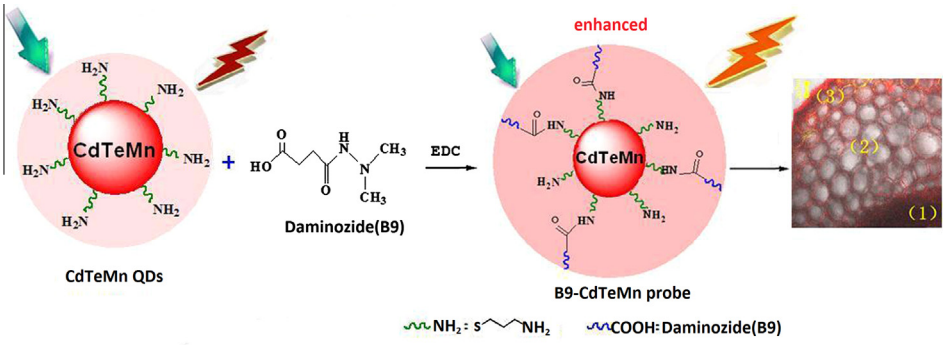
HIGHLIGHTS

- The red fluorescence of the B9-CdTeMn probe is unaffected by background interference.
- Compared with CdTe QDs, the fluorescence stability of B9-CdTeMn probe is superior.
- The B9-CdTeMn probe have the same biological activity as B9.
- The B9-CdTeMn probe is firstly used for labeling B9-binding proteins in situ.

GRAPHICAL ABSTRACT



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ABSTRACT

Daminozide (B9) is a growth inhibitor with important regulatory roles in plant growth and development. Locating and quantifying B9-binding proteins in plant tissues will assist in investigating the mechanism behind the signal transduction of B9. In this study, red fluorescent Mn-doped CdTe quantum dots (CdTeMn QDs) were synthesized by a high-temperature hydrothermal process. Since CdTeMn QDs possess a maximum fluorescence emission peak at 610 nm, their fluorescence properties are more stable than those of CdTe QDs. A B9-CdTeMn probe was synthesized by coupling B9 with CdTeMn QDs. The fluorescence intensity of the probe is double that of CdTeMn QDs; its fluorescence stability is also superior under different ambient conditions. The probe retains the biological activity of B9 and is unaffected by interference from the green fluorescent protein present in plants. Therefore, we used this probe to label B9-binding proteins selectively in root tissue sections of mung bean seedlings. These proteins were observed predominantly on the surfaces of the cell membranes of the cortex and epidermal parenchyma.

Introduction

Plant hormones behave as signaling molecules that regulate plant growth and environmental responses in various ways, with a pronounced significance in crop production. Daminozide, also known as N-Dimethylamino succinamic acid (B9), is a growth inhibitor [1]; its inhibitory mechanism has been studied to some degree. Morgan et al. found that B9 inhibited the synthesis of kaurene, thereby reducing the level of gibberellin in plants [2]. Reed et al. reported that B9 inhibited β-indole ethylamine synthesis, resulting in a reduction in indole acetic acid content in pea seedlings [3]. However, the destructive liquid extraction processes used in the above research is impossible to study the activity of B9

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in situ. The signal transduction of B9 to plant growth has not been investigated using visualization research methods, primarily because B9 has no detectable signal (such as fluorescence emission or ultraviolet absorption). The first step of signal transduction involves the binding of the plant hormone with a plant hormone receptor (binding proteins) [4]. The levels and distribution of hormone-binding proteins in plants play an important role in signal transduction. At present, there are no published studies that have used in situ visualization for labeling analysis of B9-binding proteins.

In general, there is a paucity of research on hormone binding sites or binding proteins of plants. In order to study abscisic-acid-binding sites, Kitahata et al. used biotin-labeled abscisic acid (ABA) as a probe while incubating barley aleurone protoplasts with BodipyFL fluorescein-labeled streptavidin [5]. Protoplasts that successfully bound to the probe were detected by flow cytometry. Their study observed ABA-binding sites on the plasma membranes of plant cells. In the same year, Nyangulu et al. [6] synthesized a biotinylated probe possessing structure and biological activity similar to those of abscisic acid. An affinity column filled with this probe and streptavidin-linked sepharose was then used to isolate ABA-binding proteins from rape. Since fluorescein has low resistance to photobleaching, and the separation and purification of binding proteins are difficult, the development of a convenient method for the labeling analysis of these proteins based on in situ visualization is essential.

Owing to their anti-photobleaching characteristics [7], QDs can be used as fluorescence labels to assist in the quantification and image [8] of biological molecules. Shu et al. reported ZnSe/ZnS QDs-based probes had good stability, low toxicity and biocompatibility for fluorescence imaging in a cancer model system [9]. Mattheakis et al. synthesized a probe by coupling calcium receptor G proteins with CdTe QDs to detect calcium concentrations in Chinese hamster ovary cells [10]. Doped quantum dots (d-dots) are not only as efficient as standard quantum dots, but also exhibit superior fluorescence stability under various conditions (presence of ultraviolet radiation, wide pH range), which makes these d-dots ideal as fluorescent probes in biological assays, cell and tissue research, and even in vivo investigations [11]. Manganese (II) ions can be incorporated in large proportions in VIAIIB semiconductor hosts without substantially altering the crystallographic quality of the material [12]. Geszke et al. used folic-acid-functionalized Mn-doped ZnS QDs for two-photon based fluorescence imaging of human cancer cells [13]. Huang et al. presented a "turn-on" fluorescent sensor which using the interactions between Mn-doped CdS/ZnS QDs and gold nanoparticles to detect Hg^{2+} in water samples [14].

In a previous study, we presented the successful use of a Jasmonic acid-QDs probe prepared by coupling CdTe QDs with jasmonic acid (JA) to label and image the binding sites of JA in tissues of mung bean and Arabidopsis seedling [15]. Compared to the organic dye fluorescein-5-isothiocyanate, CdTe QDs exhibit greater resistance to photobleaching. However, the green fluorescence of JA-QDs is vulnerable to interference by green fluorescence from plants. To improve the fluorescence stability of QDs and avoid such background interference, we chose B9 as a target because it also lacks any fluorescent signals. We synthesized a novel B9-CdTeMn probe based on Mn-doped CdTe QDs (CdTeMn QDs). This B9-CdTeMn probe can selectively label B9-binding proteins in plant tissues. The red fluorescence of this probe is not sensitive to background interference; meanwhile, the fluorescence stability of this probe was observed to be excellent under various conditions (ultraviolet radiation, wide pH range, ionic strength and interference from biological molecules). We used in situ visualization of this probe to accomplish the labeling analysis of B9-binding proteins.

Materials and methods

Materials and devices

Fluorescein-5-isothiocyanate (FITC), mercaptoethylamine hydrochloride (MA·HCl) was purchased from Sigma–Aldrich. $CdCl_2{\cdot}2.5H_2O$, $MnSO_4{\cdot}H_2O$ was purchased from Guangzhou Reagent (China). Tellurium powder (99.99%), $NaBH_4$, N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC·HCl), B9, D-tryptophan (D-Trp), L-tryptophan (L-Trp) were purchased from Aladdin. Ascorbic acid (Vc) was purchased from Damao Reagent (Tianjin, China). Deoxyribonucleic acid (DNA), ribonucleic acid (RNA), albumin (ALB), proline (Pro), lysine (Lys) were purchased from Ribio Biotech Co., Ltd (Shanghai, China). Lysozyme (LZM) was purchased from Oddfoni Biotechnology Reagent (Nanjing, China). All the reagents were analytical grade and used without further purification.

Transmission electron microscopy (TEM) images and Energy dispersive spectra (EDS) were performed using a TEM-2100HR microscope (JEOL, Japan). The nano-particle diameters assays were measured by Malvern Zetasizer Nano ZS nanometer particle size analyzer (Malvern, UK). XRD patterns were obtained using a D8 ADVANCE X-ray powder diffractometer (Bruker, Germany) at room temperature. Thermogravimetric analysis was conducted using a TGA 7 thermogravimetry Analyzer (TGA) (Perkin–Elmer, USA). Magnetic hysteresis loops were obtained using a MPMS XL-7 magnetic property measurement system (QUANTUM DESIGN, USA). UV–vis absorption spectra was obtained with a UV–Vis 1700 spectrophotometer (Tianmei, China). Fluorescence spectra was measured with a F-2500 spectrometer (Hitachi, Japan). Fluorescence lifetime experiments were performed by a FLS-920 combined fluorescence lifetime and steady-state spectrometer (Edinburgh, UK). The infrared spectra were recorded using Prestige-21 FT-IR spectrometer (SHIMADZU, Japan). Confocal fluorescence imaging was performed using a LSM 510 Meta Duo Scan laser scanning confocal microscope (Carl Zeiss, Germany). Fluorescence imaging was accomplished using a Nikon Eclipse 50i fluorescence microscope (Nikon, Japan).

Synthesis and purification of water-soluble CdTeMn QDs

The clear and colorless NaHTe was synthesized according to previous methods [16] with some modifications. The molar ratio of $NaBH_4$ and tellurium powder was 1:2 and the concentration of NaHTe was 0.5 M. 0.0924 g (4.0×10^{-4} mol) $CdCl_2{\cdot}2.5H_2O$, 0.1370 g (1.2×10^{-3} mol) Mercaptoethylamine hydrochloride (MA·HCl) and 0.0914 g (2.5×10^{-5} mol) $MnSO_4{\cdot}H_2O$, were first dissolved in 50 mL N_2-saturated double distilled water. The pH was then adjusted to 5.5 by adding a 1 M NaOH solution dropwise. After that, 90 μL of freshly prepared, oxygen-free NaHTe solution (4.5×10^{-5} mol) was quickly added to the mixture with vigorous stirring under N_2 atmosphere. Then the resulting solution was heated in a 50 mL Teflon-lined stainless-steel autoclave at 210 °C for 43 min and cooled with circulating water; the orange-red and transparent CdTeMn QDs solution was collected. The molar ratio of Cd^{2+}:HTe^-:Mn^{2+}:MA was 1:0.1125:0.0625:3.0 and the concentration of CdTeMn QDs solution was 9×10^{-4} M according to the dosage of HTe^-. The CdTe QDs solution was firstly dialyzed for 30 min in a refrigerator at 4 °C with dialysis bags (MWCO: 14,000 Da) to remove excess small molecules and ions. The distilled water was regularly changed every 10 min and stored at 4 °C in the dark.

Synthesis and purification of the B9-CdTeMn probe

0.0011 g (7.0×10^{-6} mol) B9 and 0.0013 g (7.0×10^{-6} mol) EDC were dissolved in 280 μL 10 mM PBS solution (pH = 5.5). After