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Fabrication of CdS Nanoparticle Coated Jasmonate Conjugates and their Interactions with Mammalian Cells

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Introduction

Self-assembled nanomaterials have been gaining importance because of their wide range of applications for the development of nanodevices (Zhang et al. 2002; Reinhoudt and Crego-Calama 2002; Zhang 2003). Molecular self-assembly primarily occurs by non-covalent interactions such as hydrogen bonding, electrostatic interactions, *van der Waals* forces and hydrophobic interactions that are the result of chemical complementarities and structural compatibility (Ratner and Bryant 2004). Depending on growth conditions, distinct structures such as micelles, vesicles, rods or tubules are formed (Hartgerink et al. 2001). There has been much focus on biological building blocks such as DNA, proteins and lipids using bottom-up approaches for the development of nanomaterials due to relatively economic, mild, and environmentally friendly methods utilized therein (Lowe 2000; Boozer et al. 2003). By combining biological building blocks with synthetic nanoparticles such as quantum dots or magnetic nanoparticles, one can prepare composites capable of a wide range of applications. For example, quantum dots (QDs) are being utilized as biomarkers for tumor targeting *in vitro* and *in vivo* (Michalet et al. 2005). QDs are slowly replacing molecular fluorophores and dyes due to their spectral stability, and high molar extinction coefficients (Leatherdale 2002). In particular, cadmium sulfide (CdS) QDs has a band gap energy of 2.52 eV (Bruchez et al. 1999). Several methods have been utilized for the growth of CdS nanoparticles such as laser ablation, electrochemical fabrication, surfactants, and, in recent times, biological templates (Artemyev et al. 1997).

In this work, we have grown CdS nanoparticles on jasmonate nanoassemblies biomimetically and examined their interactions with mammalian cells. In general, plants naturally secrete the phytohormone jasmonic acid during development and in response to biotic and abiotic stress as a defense mechanism (Traw and Bergelson 2003; Sembdner and Parthier 1993). We have developed a new class of nanomaterials by utilizing nanoassemblies of the plant phytohormone jasmonic acid (JA) as templates for the growth of quantum dots, which may have potential applications as sensors and may potentially be utilized for bioimaging applications.

Experimental Procedure

Materials

Jasmonic acid, cadmium chloride, sodium sulfide, 100µg/ml penicillin, 100 µg/ml streptomycin and 10% fetal bovine serum were purchased from Sigma Aldrich. Normal rat kidney (NRK) cells were purchased from ATCC (CRL-6509), buffer solutions of various pH values were purchased from Fisher Scientific. Dulbecco's Modified Eagle's Medium was purchased from Gibco.

Methods

JA assemblies are allowed to grow under aqueous conditions at varying pH for a period of four to six weeks at a pH range of two to nine. After formation, the assemblies are washed with deionized water and centrifuged twice at 20 000 rpm. For functionalization of the assemblies with CdS QDs, the precursor, cadmium chloride (0.1 M), is incubated with the formed JA assemblies for 48 h. The solutions are then heated to 60 °C followed by the drop-wise addition of sodium sulfide solution (0.1 M) under nitrogen. The solutions are then cooled to room temperature and centrifuged and washed thoroughly to remove unreacted materials before further analyses. Absorbance spectroscopy is carried out using a Thermo Scientific NanoDrop 2000. Readings are taken at a wavelength range of 190 nm to 600 nm. All samples are repeated in triplicate.

Fluorescence Spectroscopy is carried out using a Jobin Yvon Fluoromax 3 fluorimeter. The samples are excited at 495 nm. Each sample is analyzed in triplicate. For transmission electron microscopy (TEM), the washed samples are air-dried onto carbon-coated copper grids for characterization by TEM (JEOL 1200 EX) operating at 100 kV. The morphologies of the samples are also analyzed using scanning electron microscopy (SEM) (Hitachi S-2600N) operating between 15–25 kV. For confocal microscopy, the samples are mounted on glass slides and sealed with cover slips. The coverslips are sealed with fingernail polish and the samples are imaged with a Leica TCS-SP5 laser scanning confocal microscope.

Results and Discussion

Molecular self-assembly has attracted considerable attention for its use in the design and fabrication of nanostructures (Huck 1995). Structurally JA consists of a cyclopentane ring connected to a pentenyl group, along with one hydroxyl and a carboxyl group. The self-assembly of JA at various pH values was examined over a period of four to six weeks. In general, under acidic conditions, (pH 2-5), we observed the formation of nanospheres (figure 1a). In contrast, under neutral to basic conditions, the assembly of short fibrous structures was observed (figure 1b). It appears that under acidic conditions, hydrogen bonding interactions between the hydroxyl groups as well as the fact that the carboxyl group of JA is protonated leads to an increase in hydrogen bonding causing the formation of aggregates of spherical structures. On the other hand, under neutral to basic conditions the carboxyl group is deprotonated but hydrogen bonding still exists due to O-H group interactions. Although there is a decrease in hydrogen bonding,

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coulombic repulsions, due to the deprotonated carboxyl groups exist, causing a decrease in aggregation. Further, hydrophobic interactions between the five membered ring systems and the pentenyl groups also allow for self-assembly leading to the formation of fibrous nanostructures. Since JA is a polar acid, solvent-particle interactions also exist in addition to inter-particle attractions. These solvent-particle interactions are due to *van der Waals* interactions based on dispersion forces and dipole-induced dipole attractions (Jonas and Krüger 2002). At higher pH levels, competition between the inter-particle and solvent-particle interactions causes the assembled structures to be larger and spread out compared to the clusters formed under acidic conditions.

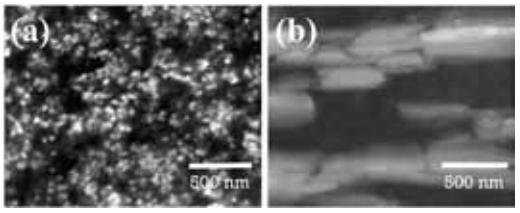
The nanoassemblies formed at pH 7 were then conjugated with CdS nanocrystals. The fluorescence spectra of CdS nanocrystals before and after conjugation with JA assemblies are shown in figure 2. Upon incorporation of JA with CdS QDs, a blue shift of 7 nm is observed compared to the peak at 540 nm for CdS nanocrystals alone indicating the incorporation of the QDs on the assemblies.

Further analysis by TEM confirmed that the JA assemblies were conjugated to CdS nanoparticles (figures 3). The CdS nanoparticles ranged between 20–30 nm in diameter and completely coated the surfaces of the JA assemblies. It is likely that the CdS nanoparticles efficiently bound to the JA assemblies due to the complex formation between the Cd+2 ions and the hydroxyl groups of the JA assemblies, followed by the formation of CdS nanoparticles. In addition, the electron diffraction pattern Fig. 3c reveals that the CdS nanoparticles bound to JA assemblies are highly crystalline.

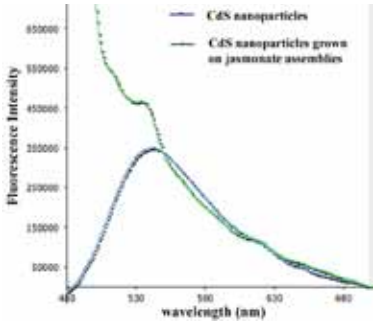
In order to explore the potential of these nanomaterials as biomarkers, the interactions of the nanocomposites with mammalian cells was explored. Confocal microscopy of the JA-CdS nanocomposites after incubation with normal rat kidney (NRK) cells is shown in figure 4. The DIC image is shown in figure 4a, while figure 4b shows the superimposition of fluorescence and DIC microscopy image indicating that the JA-CdS nanocomposites successfully adhered to the cell membranes. These results indicate that JA-CdS nanopcomposites can successfully attach to mammalian cells and may potentially be used as biomarkers.

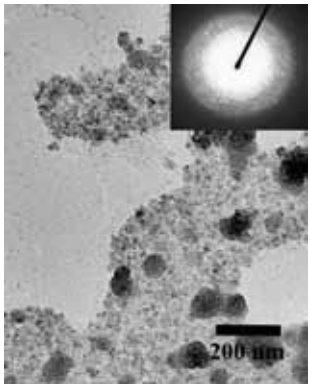
Conclusions

In conclusion, we have reported the assembly of the plant phytohormone Jasmonic Acid. We found that under acidic conditions, JA assembles into spherical nanostructures. Under neutral to basic conditions, the formation of fibrous assemblies is observed. Further, nanocomposites of JA-CdS were formed, and they were observed to be highly luminescent as indicated by confocal microscopy. The nanocomposites were found to efficiently attach to mammalian cells and may potentially be useful as biomarkers.

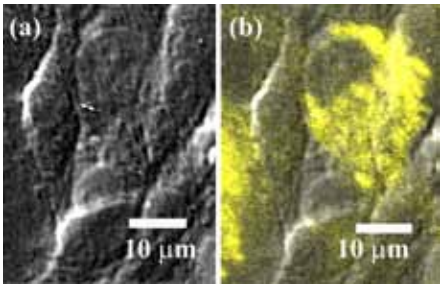


(right) **Figure 1**
(a) SEM image of jasmonate nanospheres formed under acidic conditions (pH 2); (b) SEM image of jasmonate nanorods formed under neutral conditions (pH 7). Images shown indicate growth after three weeks.





(above left) **Figure 2** Fluorescence spectra of CdS nanoparticles before and after binding to jasmonate assemblies.
(above right) **Figure 3** TEM image of CdS nanoparticles grown on the jasmonate assemblies formed at pH 7. Inset shows the diffraction pattern of the CdS nanocrystals showing the [111], [002] and [110] phases.



(right) **Figure 4**
(a) DIC image of JA-CdS nanocomposites bound to NRK cells;
(b) Overlap of DIC and Fluorescence microscopy image of JA-CdS nanocomposites in the presence of NRK cells.

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