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A novel water-based chitosan-La pesticide nanocarrier enhancing defense responses in rice (*Oryza sativa* L) growth

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ABSTRACT

To relieve the environmental pressure from overusing conventional pesticides formulations, the study of a new environmentally friendly and multifunctional formulation is so very urgent. Here, we firstly reported a lanthanum-modified chitosan oligosaccharide nanoparticles (Cos-La) prepared by a simple ionic cross-linking method to load avermectin (AVM). The loading capacity of AVM-loaded Cos-La was up to 46.3%. As a water-based formulation, Cos-La could effectively improve the persistence of AVM over 25% and reduce the photolysis rate of AVM around 20%. Furthermore, different concentrations of Cos-La were used to cultivate rice. The treated rice exhibited growth promotion effects in terms of plant height and fresh weight. With the increase in the treating concentration of Cos-La nanoparticles, the wettability of rice tended to reduce, which indicated it might lower the risk of plant diseases and pests. Further, Cos-La treated rice showed significant defense response for rice blast and the effect was two times more than equivalent Cos and $LaCl_3{\cdot}7H_2O$ mixture solution. These results showed that Cos-La not only could improve the stability and persistence of pesticides, but also could effectively promote the growth and improve the disease resistance of crops. Cos-La nanoparticles would be a promising and environmentally friendly nanocarrier of pesticides in agricultural scenarios.

1. Introduction

With continuous increase of the world population, the demand for sufficient quantity and quality of food is becoming more and more eager. It has been estimated that global food production is expected to increase 70–100% in order to feed the explosively growing population in 2050 (Tomlinson, 2013). Agricultural production continuously suffered from a large number of insect pests, diseases, and weeds leading to 40% losses to the tune of US $2000 billion per year (Peshin & Dhawan, 2009). To reduce these losses and improve productivity, the use of pesticides became necessary. According to statistical research, pesticides have enhanced the yield of total worldwide crop over 30% every year (Lamberth, Jeanmart, Luksch, & Plant, 2013). It is well-known that the use of conventional pesticides formulation is the major method to control plant diseases and insect pests (Jia et al., 2017). However, the indiscriminately overuse of conventional pesticides formulation leads to degradation of agro-ecosystems and soil health, high pesticide residue, environmental pollution (Gevao, Semple, & Jones, 2000; Wan et al., 2013) and pesticide resistance (Naqqash, Gokce, Bakhsh, & Salim, 2016). These serious damages result from not only the low efficiency of conventional pesticides formulation, but also the high use of organic solvent and surfactants in formulation, such as methylbenzene, xylene and nonylphenol surfactant (Engelskirchen et al., 2012). Thus, the development of a new water-based, release-controlled and environmentally friendly formulation is very urgent (Liu et al., 2013).

Over the past decade, the appearance of nanotechnology was potential to revolutionize agricultural practices (Scott & Chen, 1987). The use of nanoparticles in pesticide delivery had created many opportunities for safe application of conventional pesticides (Arasoglu et al., 2016; Mora-Huertas, Fessi, & Elaissari, 2010) and more and more water-based and eco-friendly nanopesticides formulations were reported lately. Those researches reports were mainly aimed at improving water dispersibility, persistence and stability of pesticides (Guan, Zhang, Tang, Wang, & Cui, 2017; Tong et al., 2017). However, there were no specific researches on whether the carriers had other biological functions such as promoting plants growth and activating defense responses to phytopathogen. Hence, we plan to prepare water-based multifunction nanoparticles in order to enhance biological functions of nanopestcides.

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It is well-know that chitosan as a natural polymer has been widely used for nano-pharmaceuticals (Hu, Wang, Li, Zeng, & Huang, 2011; Li & Huang, 2012). Nevertheless, the use of chitosan in nanocarrier of pesticides is in initial stage. In the plant system, chitosan had been reported to promote plant growth and improve multifaceted disease resistance (El Hadrami, Adam, El Hadrami, & Daayf, 2010). Besides, results of research indicated that supplying the rare earth elements similarly had beneficial effect on plant growth and disease resistance (Diatloff, Smith, & Asher, 1995). Meanwhile, the rare earth elements-modified chitosan complex had been reported for improving the bioactivity of chitosan to improve bactericidal activity (Ou, Wu, Li, Wang, & Zhang, 2013). Therefore, the rare earth elements-modified chitosan would be a more potential material for preparing multi-functional nanopesticide formulation compared with chitosan and rare earth elements.

Here, a lanthanum-modified chitosan oligosaccharide (Cos-La) nanoparticle was firstly reported. The nanoparticles were prepared by simple ionic cross-linking between chitosan oligosaccharide (Cos) and lanthanum-citric acid complex (CA-La). The avermectin (AVM) which was a typical pesticide of poor water solubility, photosensitivity and low persistence was loaded in Cos-La nanoparticles (AVM-Cos-La) successfully. The nanoparticles were evaluated by fourier transform infrared (FT-IR), X-ray diffraction (XRD), electron microscope, dynamic light scattering (DLS), energy dispersive spectrometer (EDS) and X-ray photoelectron spectroscopy (XPS) in order to study the mechanism of the formation of Cos-La and AVM loading process. Furthermore, the release-controlled ability and photostability of AVM-Cos-La were evaluated. In order to explore the other biological application of Cos-La, different concentrations of Cos-La were used to cultivate rice and then the growth situation and disease resistance of rice were investigated.

2. Materials and methods

2.1. Materials

Chitosan oligosaccharide (1500–2000 Da, 99%) with a deacetylation degree of 85.49% was purchased from the Haidebei Marine Bioengineering (Jinan) Co., Ltd. Lanthanum(III) chloride heptahydrate ($LaCl_3{\cdot}7H_2O$) was purchased from Nine-Dinn Chemistry (Shanghai) Co., Ltd. Citric acid monohydrate (CA) was obtained from RichJoint Chemical Reagents (Shanghai) Co., Ltd. Avermectin (AVM) with a purity of 96% was provided by Fengning Chemistry (Hebei) Co., Ltd. Silwet L-77 was purchased from Biotopped (Beijing) Co., Ltd. Dialysis tube (MWCO, 3500) was purchased from Yuanye Bio-Technology (Shanghai) Co., Ltd. HCl, NaOH, CH_2Cl_2 and KBr were analytical grade from Taiwei Biochemical technology (Guangzhou) Co., Ltd.

2.2. Synthesis of Cos-La nanoparticles

The CA-La solution was prepared by the following procedure. Citric acid monohydrate (105 mg, 0.5 mmol) and $LaCl_3{\cdot}7H_2O$ (37.1 mg, 0.1 mmol) were dissolved in 25 mL distilled water. NaOH (1 mol/L) and HCl (1 mol/L) solution were used to adjust the pH value to 6 and the mixed solution was agitated gently at 25 °C for 0.5 h. The transparent CA-La complex solution was obtained and the CA-La (18.3 mg) was obtained by the solid precipitating out from the CA-La complex solution.

Cos-La nanoparticles were synthesized as similar with the literature (Lin et al., 2015). In briefly, 350 mg of Cos was dissolved in 25 mL distilled water. 25 mL of CA-La complex solution was added to the Cos solution with stirring at 25 °C for 2 h. The resulting Cos-La nanoparticles (278 mg) were harvested by centrifugation (8000 r/min for 10 min), rinsed three times in distilled water and freeze-dried.

2.3. Preparation of AVM-loaded Cos-La nanoparticles

AVM-loaded Cos-La nanoparticles were prepared by the similar to above method. 350 mg of Cos was dissolved in 20 mL distilled water. After dissolving 175 mg of AVM in 10 mL of CH_2Cl_2, the organic phase was added to the Cos solution and vigorously stirred for 30 min to obtain an emulsion. At the same time, citric acid monohydrate (105 mg, 0.5 mmol) and $LaCl_3{\cdot}7H_2O$ (37.1 mg, 0.1 mmol) were dissolved in 20 mL distilled water and adjusted the pH to 6 with stirring for 0.5 h. 20 mL of complex solution was add to the emulsion over 2 h with gentle stirring at 25 °C. The AVM-Cos-La nanoparticles (247 mg) were harvested by centrifugation (8000 r/min for 10 min), rinsed several times in distilled water and freeze-dried.

To determine AVM loading efficiency (LE) and encapsulation efficiency (EE), 10 mg of freeze-dried AVM-Cos-La nanoparticles were added to 3 mL of ethanol, and the suspension subjected to ultrasonication using ultrasonic cleaner (Kunshan Ultrasonic Instruments, KQ2200DB) for 4 h to completely disassociate the AVM. The supernatant was collected by centrifugation (8000 r/min for 10 min) and then using UV–vis spectroscopy (Shimadzu, UV-2550) to determine the concentration of AVM in supernatant. The LE and EE were calculated as follows:

$$\mathrm{LE}\ (\%) = \left(\frac{\mathrm{M}}{\mathrm{M}_0}\right) \times 100\% \quad (1)$$

$$\mathrm{EE}\ (\%) = \left(\frac{\mathrm{M}}{\mathrm{W}}\right) \times 100\% \quad (2)$$

Where M is the mass of AVM in Cos-La nanoparticles, M_0 is the mass of AVM-Cos-La nanoparticle and W is the total amount of AVM used for nanoparticles preparation.

2.4. Characterization of nanoparticles

The size and morphology of Cos-La nanoparticles were determined by DLS with a Zatasizer NanoZSE (Malvern Instruments), TEM with a Tecnai 12 device (Fei, The Netherlands) and SEM using a Merlin Compact (Zeiss, Germany). The structure and compositions were characterized by FT-IR, EDS, XPS and ICP-OES. The FT-IR spectra were obtained using VERTEX 70 spectrometer (Bruker, Germany). Energy disperse spectra were determined by EDS Inca X-max (Oxford Instruments). XPS was carried out by using EscaLab 250Xi (Thermo Scientific). La element content of Cos-La nanoparticles was obtained by ICP-OES 730 (Agilent). XRD patterns of nanoparticles were obtained by UltimaIV X-ray diffractometer (Rigaku).

2.5. Controlled-release of the AVM-Cos-La nanoparticles

The drug releasing profile of the Cos-La nanoparticles was measured by the dialysis method (Zhang et al., 2017). AVM-Cos-La nanoparticles containing 5 mg of AVM were dispersed to 1 mL of ethanol/water mixture (2:1, v/v) and moved into the dialysis bags (MWCO, 3500 Da). The dialysis bag was immersed into 150 mL of ethanol/water mixture (2:1, v/v) release media in a jar. The jar was then placed onto a magnetic stirrer with gentle agitation under the room temperature. 3 mL of the release medium was taken at the predesigned interval, and the released amount of AVM was obtained by UV–vis spectroscopy. The release medium was put back to the tester after the measurement and 5 mg of pure AVM as control.

2.6. Photodegradation behavior of AVM-Cos-La nanoparticles

The photostability of the AVM-Cos-La drug-loaded system was evaluated according to the method (Liang et al., 2017). 60 mg of AVM-Cos-La nanoparticles were irradiated at room temperature by an UV lamp (254 nm, 8 W). The sample was spread out on a petri dish and the