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Synthesis and characterization of nanoparticles based on negatively charged xanthan gum and lysozyme

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ABSTRACT

Nanoparticles (XG/Ly NPs) were fabricated by like charged xanthan gum and lysozyme and the interactions between them in the preparation condition were further explored. The sizes, zeta potential, scanning electron microscope (SEM), atomic force microscopy (AFM) and X-ray photoelectron spectroscopy (XPS) were used to characterize the NPs. The interactions between them were investigated through Fourier transform infrared spectroscopy (FTIR), circular dichroism (CD), hydrophobicity and rheological analysis. The results indicated that the size-controlled (61.7 nm–108 nm) XG/Ly NPs displayed a regular spherical morphology with favorable size distribution and stability for one month. Suffering from the alkali-coupled thermal treatment, they lost their natural structures, explored more hydrophobic areas accompanied by molecule rearrangement and gelation, and then interpenetrated in forming the NPs. Ly exhibited a trend from α-helix to β-sheet transition. The NPs possess internal hydrophobic regions that barely affected by the prepared conditions. The worthwhile endeavor prepared biopolymer NPs by a negatively charged protein and polysaccharide in a simple method, and attempted to provide some useful information in physical interactions and protein/polysaccharide NP fabrications.

1. Introduction

Nanoparticulate delivery systems are expected to exert great social and environmental benefits and extensively investigated for their dimensional effects (Liu et al., 2014; Mahmoudi et al., 2011; Vinogradov, Bronich, & Kabanov, 2002). Different types of nano-sized carriers, such as polymeric nanoparticles, solid lipid nanoparticles, polymeric micelles, nanocages and dendrimers, are being developed for various delivery applications (Gaspar & Duncan, 2009; Longmire, Choyke, & Kobayashi, 2008). Polymeric nanoparticles can be fabricated from polysaccharides, proteins and their synthetic polymers (Mizrahy & Peer, 2012). Considering their bio-toxicity, natural proteins and polysaccharide usually become the first choice for preparing NPs in a green procedure. Systems based on β-casein (Pan, Yu, Yao, & Shao, 2007), β-lactoglobulin (Jones, Decker, & McClements, 2009), bovine serum albumin (Jun et al., 2011), cholesteryl pullulan (Morimoto et al., 2013), sodium carboxymethyl cellulose (Chang & Zhang, 2011), pectin (Chittasupho, Jaturanpinyo, & Mangmool, 2013) and chitosan (Pramanik, Laha, Pramanik, & Karmakar, 2013) have been studied for delivering drugs, nutrients and bioactive substances.

It is widely regarded that electrostatic and hydrophobic interactions were the main driving forces in NP fabrication. Moderate charge attraction and thermal treatment favor their formation (Hosseini et al., 2013). However, most previous studies concentrated on polysaccharide/protein NPs with opposite-charges. It was easy for the oppositely-charged polymers to form a complex in appropriate conditions. To obtain stable NPs, the electrostatic and hydrophobic interactions reach a balanced state to sustain the NPs with low free energy (Coelho et al., 2011; Ye, 2008). Our research group has also combined lysozyme with sodium carboxymethyl cellulose, κ-carrageenan and pectin to make vehicles for drug and nutrient controlled release (Li et al., 2014; Lin et al., 2015; Xu et al., 2014). However, there are rare researches on polysaccharide/protein NPs prepared by like-charges and the interactions between them.

Lysozyme and xanthan gum are the most common food ingredients. The blend solutions become flocculate for strong electrostatic interactions at any pH value when they carry opposite charges. Only in the like charged condition could they form favorable NPs. This special phenomenon roused our great interest, and therefore, in this paper, XG/Ly NPs were first prepared when both of the biopolymers were negatively charged. The obtained NPs were subsequently characterized by size and SEM, AFM and XPS. To explore the interactions between the two polymers, FTIR, CD, hydrophobicity and rheological analyses were conducted in a simulated condition. The conformational changes of Ly during the treatment in the absence and presence of XG were also enriched. This interesting attempt may provide some useful information in physical interactions and NP fabrications.

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2. Materials and methods

2.1. Materials

Xanthan gum (XG) was purchased from Shanghai Source Biological Technology Co., Ltd. Lysozyme (Ly, Mw = 14.3 kDa) from chicken egg white was obtained from the National Medicine Group Chemical Reagent Co., Ltd. Other chemicals were of analytical grade and used without purification. All the solutions in the experiments were prepared using ultrapure water through a Millipore (Millipore, Milford, MA, USA) Milli-Q water purification system.

2.2. Preparation of XG/Ly NPs

Stock XG and Ly solutions with a concentration of 1.0 mg/mL were prepared by gentle magnetic stirring at room temperature for 6 h and 2 h, respectively. The pH of the mixture was adjusted to 11.8. After 1 h stirring, the mixtures were immediately heated for a period of time. The effect of different weight ratios (3:1, 2:1, 1:1, 1:2 and 1:3), temperatures (40–90 °C) and heating times (5–60 min) on the XG/Ly NP formation were researched.

2.3. Characterization of XG/Ly NPs

Size and zeta potential (ζ) were measured by a commercial laser light scattering instrument (Malvern ZEN3690, Malvern Instruments) at 25 °C. Morphology of the NPs was investigated using a scanning electron microscope (SEM, JSM-6390LV, Japan). Initially, the vacuum dried samples were coated with about 20 nm gold–palladium under argon atmosphere for 30 s using a gold sputter module in a high vacuum evaporator. Atomic force microscopy (AFM, Agilent 5500) was further used to measure the morphology of NPs in tapping mode. X-ray photoelectron spectroscopy (XPS) was recorded with a VG Multilab-2000. The Fourier transform infrared spectra (FT-IR) were recorded with a Nicolet Nexus 470 spectrometer with air black and 4 cm^{-1} resolution.

2.4. Stability of XG/Ly NPs

The sample or samples were stored at 4 °C and the hydrate particle size was measured after a period of time. Additionally, 0.1% SDS, 100 mM NaCl and urea were added into the NP solutions for stability evaluation.

2.5. Circular dichroism analysis

The Ly solutions (0.1 mg/mL) with different pH (3.0–11.8) were recorded in a 0.1 cm quartz cuvette from 240 to 195 nm at 25 °C using a Jasco J-1500 Circular Dichroism Spectrometer (JASCO, Tokyo, Japan) purged with N_2. Furthermore, 0.1 mg/mL Ly solution and mixed with XG, sharing the same concentration, were scanned after heat treatment (25 °C–80 °C) for 5 s. The CD spectrum results were obtained by repeating three scans (bandwidth, 1 nm, scan speed 50 nm/min).

2.6. Hydrophobicity analysis

The hydrophobicity of the NPs prepared in different conditions was evaluated by fluorescent probe techniques using pyrene as a hydrophobic fluorescent probe (Payyappilly, Dhara, & Chattopadhyay, 2014). Pyrene solution was added into the XG, Ly and XG/Ly NP solutions (2×10^{-7} g/mL) prepared at different ratios and temperatures. The mixtures were gently magnetically stirred for 12 h in the dark. The fluorescent spectra were measured using a fluorescence spectrophotometer (F-4600 FL, Hitachi Corp., Japan) at room temperature before the samples were stored at 4 °C for another 36 h. Excitation was carried at 335 nm and emission spectra ranging from 360 nm to 420 nm were recorded.

2.7. Rheological analysis

Dynamic viscoelasticity of the XG/Ly mixture was conducted using an AR2000ex rheometer (TA, UK) with a parallel plate (diameter 40 mm, gap 1.0 mm). The storage modulus (G′) and loss modulus (G″) as

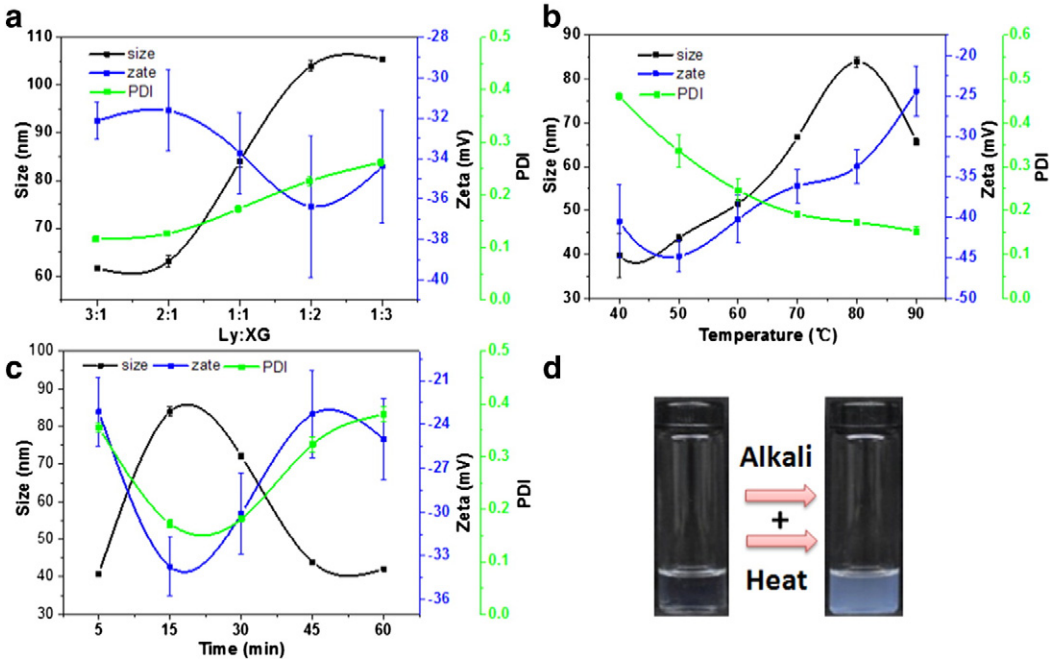

Fig. 1. Size, PDI and zeta potential of XG/Ly NPs prepared with different Ly/XG ratios at 80 °C for 15 min (a), different incubation temperatures with a Ly/XG ratio of 1:1 for 15 min (b) and incubation time with Ly/XG ratio of 1:1 at 80 °C (c). The mixture before and after treatment with alkali coupled heat (d).