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Environment induced self-aggregation behavior of κ-carrageenan/lysozyme complex

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ABSTRACT

Self-aggregation behaviors of κ-carrageenan/lysozyme (CRG/Ly) complex inducing by intrinsic and extrinsic factors were investigated form macro and micro aspects, including the ratio, pH, temperature and salt. High Ly content promote aggregation behavior of CRG/Ly complex, while CRG played a suppressive role. Except for CRY5, the complex displayed nano-size distribution. Both CRG addition and the self-aggregation behavior could enhance the thermal stability of Ly. Low ζ-potential induced by pH made the CRG/Ly complex easy to self-aggregate into structure with large size due to the gradual association of complex to form lager interpolymeric complexes. The tunable process means to control the dissociation and association behavior between interpolymeric complexes and soluble complex by pH. All systems shared high NaCl tolerance and presented stable transmittance. Heat-induced self-aggregation exhibited a temperature and polymers ratios dependant manner. The mechanisms of environment induced self-aggregation into interpolymeric complexes reflected the re-balanced physical interaction when they suffered from any treatment. Controlling and regulating self-aggregation behavior of protein/polysaccharide system could enrich the soft structures from food respective and also provide practical theory for food process.

1. Introduction

Polyelectrolyte complexes, preparing by mixing solutions of oppositely charged polyelectrolytes, have has been found for many years (Laneuville, Turgeon, Sanchez, & Paquin, 2006; Thünemann, Müller, Dautzenberg, Joanny, & Löwen, 2004). Protein and polysaccharide, two common natural polyelectrolyte, are known to have complicated interactions and hard to control (Evans, Ratcliffe, & Williams, 2013; Luo & Wang, 2014). Their complexation is always the active field of research in the past decade because their simultaneous use commonly occurs in food products. And their interactions play a significant role in regulating the structure, texture, and stability of food systems (Doublier, Garnier, Renard, & Sanchez, 2000; Schmitt & Turgeon, 2011; Tran & Rousseau, 2013). The protein/polysaccharide complexes have been widely used as friendly vehicle to control release of proteins and drugs (Liu et al., 2013; Salmaso & Caliceti, 2013), to encapsulate bioactive and other compounds (Yang, Wu, Li, Zhou, & Wang, 2013), and to fabricate structured organization (Gosal & Ross-Murphy, 2000).

However, the instability of complexes formed by oppositely charged protein and polysaccharide is an inevitable problem. Electrostatic interaction is the prevalent primary interaction controlling the complexation of proteins/polysaccharides, which can also be affected by many extrinsic factors, such as ionic strength, pH, protein/polysaccharide ratio and thermal treatment (Li et al., 2012; Schmitt & Turgeon, 2011). Any external disturbances may induce phase transition. Therefore, there is a need to understand the self-aggregation behavior induced by environmental conditions for better controlling this transition. Although many complex systems have been widely studied that mainly focused on the milk protein, vegetable proteins, cereal protein, egg protein and other protein with anionic or cationic polysaccharides (Hosseini et al., 2013; Lacroix & Li-Chan, 2014; Teng, Luo, & Wang, 2013; Luo, Zhang, Whent, Yu, & Wang, 2011). The intrinsic factors of each proteins/polysaccharides system, such as molecular weight and charge density, determined that each associative behavior was different with other systems (Schmitt & Turgeon, 2011).

Both the two biopolymers of lysozyme and κ-carrageenan are extensively used in food and medicinal application. CRG is a kind of sulfated linear polysaccharide extracted from red seaweed, and

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constitute of galactose and anhydrogalactose units which are linked by glycosidic bonds (Rodrigues, da Costa, & Grenha, 2012). CRG is a kind of bioactive polysaccharides and dietary fiber with strong ionic properties for half-ester sulfate group, and therefore has a high capacity to interact with proteins. Ly, the main protein component of the egg white fraction, has a molar mass of 14.3 kDa and been received attentions for its use as a kind of food preservative (Schuh, Schwarzenbolz, & Henle, 2010). As our group reported before the two biopolymers with opposite charges could easily self-assemble into soluble complex in neutral condition (Xu et al., 2014), But the self-aggregation behavior of the system induced by environment has also not been reported.

Therefore, in the present work, we investigated self-aggregation behavior of CRG/Ly complex induced by intrinsic and extrinsic factors, including initial CRG/Ly ratio, pH, ionic strength and heat treatment. The transmittance, size, ζ-potential, micro-structure scanning electron microscope (SEM) and transmission electron microscopy (TEM) were used to monitor the association from optical images and micro structure. The purpose of this study was to evaluate the effects of pH, NaCl concentrations, the CRG/Ly ratios and thermal treatment on the self-aggregation behavior and the environmental tolerance of the complexes.

2. Materials and methods

2.1. Materials

Lysozyme (Ly, Mw = 14.3 kDa) from chicken egg white was obtained from Sinopharm Chemical Reagent co., LTD, and κ-carrageenan (CRG) was purchased from Aladdin Chemistry Co. LTD. Other chemicals were reagent grade and used without purification. All the solutions used in the experiments were prepared using ultrapure water through a Millipore (Millipore, Milford, MA, USA) Milli-Q water purification system.

2.2. Preparation of complexes

Ly was dissolved in purified water with gentle magnetic stirring for 2 h at room temperature at the concentration of 1.0 mg/mL. The same concentration of CRG solution (1.0 mg/mL) was prepared by stirring the solution at 70 °C for 30 min for complete dissolution. The two biopolymers were mixed with different weigh CRG/Ly ratios of 3:1, 2:1, 1:1, 1:2 and 1:3. The resulted mixtures were defined as CRY1, CRY2, CRY3, CRY4 and CRY5, respectively. Additionally, the effects of pH, salt and heat treatment on self-aggregation behavior of the CRG/Ly complex were further investigated.

2.3. Size and ζ-potential measurements

The nano/micro size and ζ-potential measurements were analyzed by dynamic light scattering (Nano-Z, Malvern Instruments, UK) and a Laser Particle Size Analyzer (Malvern 2000, Malvern Instruments, UK). The complexes were directly measured at 25 °C and the ζ-potential was determined from the manufacturer's software (version 6.34). Each measurement was performed in triplicates.

2.4. Transmittance measurements

Transmittance of the CRG/Ly system was measured at 600 nm as described in previous method at different condition (Lin, Chen, & Liu, 2009), including different pH (4.0–9.0), salt concentration (0–25 mM) and heat treatment (60 °C and 80 °C for 30 min). The purified water was chosen as the blank at the same condition.

2.5. Differential scanning calorimetry measurement

Differential scanning calorimetry (DSC) experiments were performed using a 204-F1 (Netzsch, Germany). The freeze-dried CRG/Ly mixtures and Ly (2–5 mg) with 10 μL of water were placed in an aluminum pans. Then the aluminum pans were sealed and equilibrated at 25 °C for 24 h (Kumar, Ganesan, Selvaraj, & Rao, 2014). The samples were measured at the scanning rate of 5 °C/min with the temperature ranging from 40 °C to 110 °C. Another aluminum pan without sample was chosen as a reference.

2.6. Morphological observation

Scanning electron microscope (JSM-6390LV, Japan) and transmission electron microscopy (H-7650, Hitachi, Japan) were used to further intuitively observe the morphology of the self-aggregation. Before SEM experiment, the vacuum freeze dried CRG/Ly mixtures were coated with about 20 nm gold-palladium under argon atmosphere using a gold sputter module in a high vacuum evaporator. The sputtered time was about 30 s and the accelerating voltage was 15 kV. For TEM test, a drop of CRG/Ly solution was dispensed directly onto a carbon coated copper grid and allowed to dry automatically in a vacuum desiccator. Then the pictures of the prepared samples were obtained with desired magnification.

3. Results and discussion

3.1. Aggregation behavior

Aggregation behavior was common phenomenon for protein/polysaccharide systems. But the behavioral properties were different and determined by intrinsic and extrinsic factors. The polymer molecules with different molecular weight and charge density intrinsically decided the complex formation and its phase transition. For CRG/Ly system, the complex formed automatically without any treatments. The size variation of the complex with the ratios of CRG/Ly illustrated the aggregation behavior (Fig. 1). The average size increased as the ratios of CRG/Ly increased. The increasing rate was slow first, and then increased dramatically as the Ly contents were further increased. The first increase was beneficial to the higher Ly content and the lower densities of CRG/Ly system that endowed the Ly could efficiently combine with CRG. The high negative charges obtained from CRG could further help to sustain the system stability. At high Ly concentration, more Ly could be available for its physical interaction with CRG thus making a CRG/Ly complex. Additionally, CRG/Ly systems with

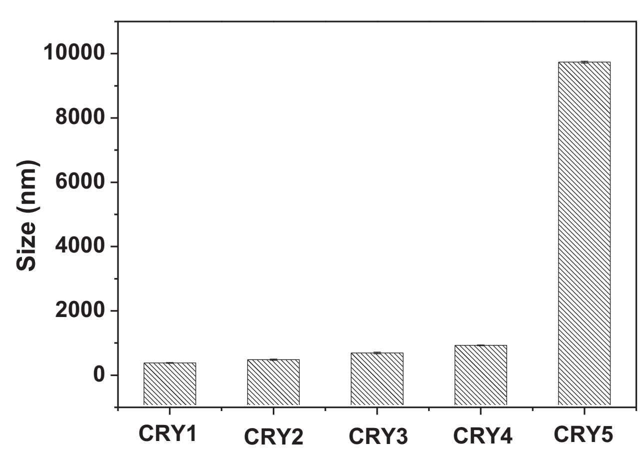

Fig. 1. The size of CRG/Ly system (natural pH about 6.5) with different ratios.