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Ovalbumin-chitosan complex coacervation: Phase behavior, thermodynamic and rheological properties

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ABSTRACT

This paper investigated the influence of pH, ionic strength, and the ratio of protein/polysaccharide on the interaction between ovalbumin (OVA) and chitosan (CS) through turbidity, zeta potential, confocal laser scanning microscope (CLSM) and isothermal titration calorimetry (ITC). Dynamic rheometer was used to study rheological properties of the complex coacervates. Results of phase diagram and microstructure showed that complexes and coacervates were formed at specific pH values via the electrostatic interactions. The lower level of ionic strength (C_{NaCl}≤200 mM) promoted the formation of OVA/CS coacervates, while higher ionic strength (C_{NaCl}>200 mM) inhibited the formation of coacervates due to the screening of macromolecular charges by salt ions. Additionally, the increase of OVA/CS ratio from 1:1 to 10:1 caused the decrease of critical pH (pH_c and pH_φ). The interaction between OVA and CS was spontaneous exothermic process at pH 4.0 and pH 5.5 according to ITC measurement. The affinity constant and stoichiometric ratio between OVA and CS at pH 5.5 was much higher than that of pH 4.0. It indicated that the change of binding energy along with OVA titration depended on the pH. Moreover, dynamic rheological analysis results showed that the lower salt concentration (C_{NaCl}≤50 mM) and OVA/CS ratio (from 1:1 to 3:1) promoted the formation of coacervates structure, which had a stronger gel strength. However, the higher salts and OVA/CS ratio (from 3:1 to 10:1) (C_{NaCl}>50 mM) led to weaker elasticity of OVA/CS coacervates.

1. Introduction

As essential components in food system, proteins and polysaccharides normally co-exist. They impact texture, structure and stability of food through the behavior of thickening or gelling and the properties of surface (Doublier, Garnier, Renarda, & Sanchez, 2000; Zhao et al., 2015). Furthermore, due to the specific structure, size and composition, protein–polysaccharide complexes and coacervates exhibited a wide variety of functionalities for the development of complex food products (Schmitt & Turgeon, 2011). Therefore, studies on interactions between proteins and polysaccharides have been trending up steadily in the past decades (Sarika, Pavithran, & James., 2015).

In aqueous solutions, formation of protein-polysaccharide complexes is driven by electrostatic interactions between oppositely charged macromolecules (Schmitt & Turgeon, 2011). Additionally, hydrogen-bonding, hydrophobic and steric interactions also exist (de Kruif, & Tuinier, 2001). The electrostatic interactions between proteins and polysaccharides can be divided into thermodynamic incompatible phase and thermodynamic compatible phase, which are affected by the solution external conditions and the molecular properties (Grinberg & Tolstoguzov, 1997; Hadian et al., 2016; Schmitt & Turgeon, 2011). In addition, the phase behavior and stability of the complex system are also influenced by temperature and mechanical factors (shearing rate, pressure, time) (Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998).

Complex coacervation of protein-polysaccharide mixtures is being mostly driven by charge neutralization (Schmitt & Turgeon, 2011). With the decrease of pH, the mixture system of protein and anionic polysaccharide go through the formation of (i) soluble

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complexes (at pH > pH_c), (ii) soluble and insoluble complexes (at $pH_{\varphi1}$<pH < pH_c), (iii) coacervates after macroscopic phase separation (at $pH_{\varphi2}$<pH < $pH_{\varphi1}$) and (iv) soluble state due to the protonation of polysaccharide (pH < $pH_{\varphi2}$) (Turgeon, Beaulieu, Schmitt, & Sanchez, 2003). Generally, charge screening, which is induced by high amounts of salts in solution, reduced the electrostatic interaction and inhibited the formation of complex. However, some studies have reported that the addition of a certain amount of salt ions promoted the formation of protein-polysaccharide complex (Laos, Brownsey, & Ring, 2007; Seyrek, Dubin, Tribet, & Gamble, 2003; Wang, Lee, Wang, & Huang, 2007; Wang, Wang, Ruengruglikit, & Huang, 2007; Weinbreck, de Vries, Schrooyen, & de Kruif, 2003; Weinbreck, Nieuwenhuijse, Robijn, & de Kruif, 2003). Furthermore, the protein-to-polysaccharide ratio impacts the formation of complex coacervation by changing the charge balance. If protein or polysaccharide is excessive, soluble complexes are formed owing to the existence of no neutralized charges (Schmitt et al., 1998).

Ovalbumin (OVA), the major protein in egg, predominantly affects the functional properties of egg white protein (Mine, 1995). In order to meet the requirements of various kinds of applications in food industry, complexion of ovalbumin-polysaccharide systems have received intensive research interest recently. Several studies reported the interaction between ovalbumin and polysaccharide (Niua et al., 2014; Niu et al., 2015; Souza & Garcia-Rojas, 2015). Chitosan (CS) is the second most abundant polysaccharide in the nature that carries positive charges in acidic solution, which was commonly used as thickening and coating agents in food industry (Huang, Sun, Xiao, & Yang, 2012; Menchicchi et al., 2014). Yu et al. had prepared stable and pH-sensitive nanogels via self-assembly technique of chitosan and ovalbumin (Yu, Hu, Pan, Ping Yao, & Jiang, 2006). Until now the complex coacervation of OVA and CS has not yet been systematically studied. Therefore, in this study the phase behavior of OVA/CS complex coacervation as a function of pH, iron strength and OVA/CS ratio were investigated by turbidity measurements. The parameters of binding constant, enthalpy, entropy, and binding stoichiometry of interactions during this thermodynamic process were quantified with isothermal titration calorimetry (ITC). Additionally, the effects of salt concentration and initial OVA/CS ratio on the rheological properties of OVA/CS coacervates were investigated.

2. Materials and methods

2.1. Materials

Ovalbumin (OVA, A5503, >98% pure by agarose electrophoresis, and a molecular weight of 45 kDa) was purchased from Sigma Co. (St. Louis, MO) without further purification. Chitosan (CS, deacetylation degree about 90.5%, and the molecular weight was about 350 kDa) was purchased from Qingdao Yunzhou Biochemistry Co., Ltd. (Shandong, China). Other reagents were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All reagents were analytical grade unless otherwise stated.

2.2. Preparation of OVA/CS aqueous mixtures

OVA (1%, w/v) stock solution was obtained by dissolving powder in deionized water under gentle stirring (300 rpm) at room temperature for 2 h and then overnight at 4 °C to ensure protein dissolution. Moreover, sodium azide (0.02%, w/v) was added to inhibit bacteria growth. CS (1%, w/v) stock solution was obtained by dissolving powder in acetic acid solution (1%, w/v) and stirring intensively (1000 rpm) at room temperature overnight. OVA/CS mixtures with weight ratios of 1:1, 3:1, 5:1, 7:1 and 10:1 were obtained by mixing corresponding stock solutions.

2.3. Turbidimetric titrations

The turbidity was measured using UV–vis spectrophotometer (UV-1100, MAPDA) at 600 nm and the pH was monitored using a pH meter (Mettler Toledo PE20). The transmittance (T) of spectrophotometer was calibrated with deionized water to 100% and the turbidity of samples was reported as 100-T%. OVA/CS mixtures were prepared by adjusting pH value to 2.8 with 1 M of HCl under magnetic stirring and total content of biopolymers was fixed as 0.5% (W/V). Sodium hydroxide solution (0.1 M, 0.5 M and 1 M) was used to adjust the pH of OVA solution, CS solution and the mixture of OVA/CS. All measurements were conducted at 25 °C and repeated three times.

2.4. Zeta potential measurement

Zeta potential was determined using ZS Zetasizer Nano (Malvern Instrument Ltd., UK). Investigated solution was filled into a zeta potential folded capillary cell. The instrument determined the zeta potential with the Henry equation (Yuan, Wan, Yang, & Yin, 2014). All measurements were carried out at 25 °C and repeated three times.

2.5. Isothermal titration calorimetry (ITC)

The thermodynamic parameters of OVA/CS complexs under the impact of pH 4.0 and 5.5 were investigated by using ITC200

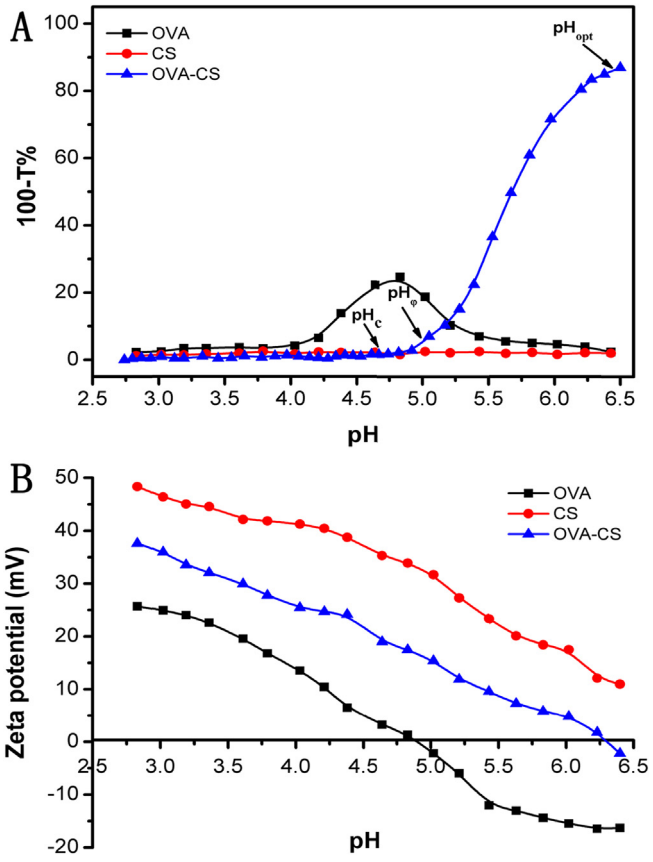

Fig. 1. Turbidity curves (A) and zeta potential (B) of OVA/CS mixtures as a function of pH at NaCl concentration (C_{NaCl}) = 0 mM. The ratio of OVA: CS = 3:1 (w/w), and the total biopolymer concentration is 0.5% (w/v).