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Phase separation behavior and structural analysis of ovalbumin–gum arabic complex coacervation

Fuge Niu [a,b], Yiting Dong [a,b], Fei Shen [a,b], Jiaqi Wang [a,b], Yuntao Liu [a,b], Yujie Su [a,b], Rongrong Xu [a,b], Junwei Wang [c], Yanjun Yang [a,b,*]

[a] State Key Laboratory of Food Science and Technology, Jiangnan University, No. 1800 Lihu Avenue, Wuxi, Jiangsu 214122, PR China
[b] School of Food Science, Jiangnan University, No. 1800 Lihu Avenue, Wuxi, Jiangsu 214122, PR China
[c] Kang De Biological Products Co., Ltd. Nantong, Jiangsu 226611, PR China

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ABSTRACT

Phase separation behavior and structure between ovalbumin (OVA) and gum arabic (GA) were investigated at pH 1.5–6.5; 2:1 OVA:GA mixing ratio, total biopolymer concentration 0.05 wt%. Soluble and insoluble complexes were observed as a function of pH. The biopolymers particles had a tiny diameter ($d \leq 500$ nm) at pH above 4.0 or below 2.5, and increased sharply at pH 4.0 together with the turbidity under the same conditions. As the biopolymers mixing ratios increased from 1:3 to 24:1, critical pHs shifted towards higher pH. However, monovalent ions had a same influence on OVA–GA complex formation; the divalent cations reduced the formation of complex compared with same concentration monovalent ions. Second-derivative UV spectra and intrinsic fluorescence results indicated that the unfolding of the tertiary conformation was induced by GA, and more tryptophan residues were buried inside the OVA protein after complexation.

1. Introduction

Proteins and polysaccharides are the essential functional ingredients determining the texture, structure and shelf-life of most food products (Dickinson, 2008; Doublier, Garnier, Renard, & Sanchez, 2000). In general, the formation of protein–polysaccharide complexes coacervates and phase separation behavior are affected by physicochemical parameters, such as solvent conditions (e.g., pH and salts) and the biopolymers characteristics (e.g., mixing ratio, concentration, charge density, type, chain length, reactive groups, branching, molecular weight, and so on). Various degrees of interactions can occur, leading in some cases to phase separation (Schmitt & Turgeon, 2011; Turgeon, Beaulieu, Schmitt, & Sanchez, 2003; Weinbreck, De Vries, Schrooyen, & De Kruif, 2003). Interactions between food macromolecules can display one of the three following behaviors: co-solubility, thermodynamic incompatibility and complex formation (De Kruif & Tuinier, 2001; Vivian & Callis, 2001). For dilute solutions the mixture is stable because mixing is driven by entropy and biopolymers are co-soluble (De Kruif & Tuinier, 2001; Turgeon et al., 2003). The system may become unstable with increasing the biopolymers concentration. Segregative phase behavior arises when both biopolymers are incompatible and repel one another (Elmer, Karaca, Low, & Nickerson, 2011). Associative phase separation occurs when biopolymers carry an opposing charge and electrostatic attraction occurs, leading to separation into biopolymer-rich and solvent-rich phases (De Kruif, Weinbreck, & de Vries, 2004; Doublier et al., 2000).

In the previous decades, protein/polysaccharide complexes and coacervates have received increasing research interest in order to broaden the possible food applications (Weinbreck, Minor, & De Kruif, 2004; Weinbreck, Nieuwenhuijse, Robijn, & De Kruif, 2004; Weinbreck, Nieuwenhuijse, Robijn, & De Kruif, 2003). An increasing number of biopolymers pairs has been investigated, enabling to identify the most important physicochemical parameters controlling complex formation (Anal, Tobiassen, Flanagan, & Singh, 2008; Gilsenan, Richardson, & Morris, 2003; Lam, Paulsen, & Corredig, 2008; Laneuville, Paquin, & Turgeon, 2000; Liu, Low, & Nickerson, 2009; Singh & Burgess, 1989; Weinbreck, Nieuwenhuijse, et al., 2004). It is widely believed to be associated with two pH-induced structural transition events, corresponding to the formation of both soluble and insoluble complexes (Ducel, Richard, Saulnier, Popineau, & Boury, 2004; Sanchez, Mekhloufi, & Renard, 2006). The critical pH boundaries pH_c, $pH_{\varphi 1}$ and $pH_{\varphi 2}$ are

* Corresponding author. School of Food Science, Jiangnan University, No. 1800 Lihu Avenue, Wuxi, Jiangsu 214122, PR China. Tel./fax: +86 0510 85329080.
E-mail addresses: niufg123@hotmail.com (F. Niu), yangyj@jiangnan.edu.cn (Y. Yang).

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determined. The formation of soluble protein–polysaccharide complexes is initiated at pH_c, which precede the pH of visual phase separation called $pH_{\varphi 1}$. Complete dissolution of complexes occurs at $pH_{\varphi 2}$, as both protein–polysaccharide carry a similar net charge (De Kruif & Tuinier, 2001; Elmer et al., 2011; Mekhloufi, Sanchez, Renard, Guillemin, & Hardy, 2005; Turgeon et al., 2003). Kinetics of phase separation of protein/polysaccharide complexes and coacervates are also investigated. Weinbreck et al. confirmed the very fast coarsening (0.3 mL/min) of the whey protein isolate–gum arabic mixture at pH 4.2 and 3 wt%. Sanchez et al. (2006) concluded that the coarsening mechanism was a nucleation and growth one through controlling in situ acidification enabled to access the initial steps of phase separation. Nevertheless, food systems are complex and therefore the mechanism of phase separation and structure of the complexes and coacervates should be investigated with a variety of experimental techniques (Mekhloufi et al., 2005).

The nature biopolymers used in this work are ovalbumin (OVA) and gum arabic (GA). Chicken egg ovalbumin is the main constituent of egg white protein (~65%), the molecular mass of ovalbumin is 42.7 kDa, and it is an important food ingredient with structural functionality including emulsifying properties and foam stability. Egg white proteins are extensively utilized in processed foods (Choi, Kim, Park, & Moon, 2005). Gum arabic is a complex polysaccharide exuded from the African tree *Acacia senegal*. It is an anionic arabinogalactan polysaccharide–protein complex, composed of three fractions. Fraction 1, which represents 88.4% of the total, is an arabinogalactan with molecular mass 2.79×10^5 and is deficient in protein. Fraction 2, which represents 10.4% of the total, is an arabinogalactan–protein complex with a molecular mass of 1.45×10^6, containing ~50% of the total protein. It is envisaged that on average each molecule of fraction 2 consists of five carbohydrate blocks of molecular mass $\sim 2.8 \times 10^5$ covalently linked through a chain of amino acid residues. Fraction 3 represents only 1.24% of the total gum but contains ~25% of the total protein and has been shown to consist of one or more glycoproteins (Randall, Phillips, & Williams, 1989).

The aims of this work are to investigate the complex formation of OVA and GA as a function of ratios and different ionics, and clarify the mechanism of interaction of biopolymers and determine the effect of GA on the structure of OVA by using second-derivative UV spectra and intrinsic fluorescence spectrometry during complex coacervation. The results could also be used for practical applications, enabling the design of complex food systems based on complexes and coacervates (DeMars & Ziegler, 2001; Norton & Frith, 2001; Tolstoguzov, 1995).

2. Materials and methods

2.1. Materials

Ovalbumin (lyophilized powder, molecular weight 42.7 kDa) was purchased from Amresco Chemical Co. (Boise, USA). According to the report, the composition of the powder was 90.3% total protein (% N × 6.25), 5.92% moisture, 0.2% fat and 2.82% ash.

Gum arabic was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) .The powder contained 9.2% moisture, 89.24% dry solid, and 4.91% ash (*w*/*w*). All the chemicals used were of analytical grade and were purchased from either Sigma Chemical Co. (St. Louis, MO, USA) or Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Sample preparation

Various concentrations of OVA and GA mixtures were obtained by dissolving each powder in deionized water under gentle stirring (500 rpm) at room temperature for 4 h and then overnight at 4 °C to ensure biopolymer dissolution. OVA and GA solutions were adjusted to pH 8.0 using 0.1 M NaOH before further mixing operations. Mixtures of OVA and GA were prepared by mixing appropriate masses of stock solutions. The mixture was acidified by the addition of HCl (0.05, 0.5, 1, 2 M). Dilution effects and changes to solution conductivity were considered to be minimal, as described by Liu et al. (2009). Critical pH values (pH_c, $pH_{\varphi 1}$, pH_{opt}, $pH_{\varphi 2}$) associated with structure-forming events were determined graphically according to the methods of Weinbreck, Nieuwenhuijse et al. (2004). The effects of the OVA:GA (4:1–24:1, *w*/*w*), the salt types, the temperature (4–55 °C) and the total biopolymer concentration (0.05–3.0% *w*/*w*) on complex formation were researched.

2.3. Turbidimetric analysis

The turbidity of mixtures of OVA and GA was determined from the absorbance using an UV/Vis spectrophotometer (WFJ 2000, UNICO, St. Louis, USA) with a 1 cm path length optical probe at 600 nm. All measurements were conducted at 25 °C and the turbidity was then measured as a function of pH. The turbidity (T) was defined as

$$(T) = -\ln(I/I_0)$$

where I is the optical density that passes through a volume of solution of 1 cm length and I_0 is the incident light intensity. All samples were analyzed in triplicate. Control measurements with only OVA and only GA were systematically carried out under the same conditions as the mixtures of biopolymers.

2.4. Particle size distribution determination

The sample used for turbidimetric analysis as a function of pH was also applied for the measurement of particle size. The particle size changes of complex were also characterized by using a Brookhaven Instruments ZetaPALS (Holtsville, NY) every 2 min for up to 20 min at a constant concentration (0.05% *w*/*w*) and ratio (OVA:GA 2:1, *w*/*w*). Default settings on the ZetaPALS were used, i.e. dielectric constant, refractive index and viscosity were assumed to be the same as for water, and the *Smoluchowski* approximation was used. 2 mL sample were prepared and measured at 25 °C at a fixed angle of 90°. The particle size measurement was done in triplicate for each sample.

2.5. Electrophoretic mobility

Electrophoretic mobility (U_E) for homogenous and 2:1 OVA:GA mixture solutions were investigated as a function of pH (6.50–1.50) using a Zetasizer 2000 (Malvern Instruments Ltd., Malvern, UK). All samples were prepared at a total biopolymer concentration of 0.05% (*w*/*v*), measured at room temperature (21–23 °C) and were prepared and acidified using a concentration gradient of HCl solutions as previously described. Measurements were taken every 0.5 pH increments between pH 6.50 and 1.50. Using the Henry equation, the U_E was used to give an estimate of the zeta potential (ζ), which gave an estimate of the surface charge on the biopolymer

$$U_E = 2\varepsilon\zeta f(\kappa\alpha)/(3\eta)$$

where η is the dispersion viscosity, ε is the permittivity, and $f(\kappa\alpha)$ is a function related to the ratio of particle radius (α) and the Debye length (η). Using the Smoluchowski approximation $f(\kappa\alpha)$ equaled 1.5. All measurements were made in triplicate.