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Mutual titration of soy proteins and gum arabic and the complexing behavior studied by isothermal titration calorimetry, turbidity and ternary phase boundaries

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ABSTRACT

Complexing between soy proteins (SP) and gum arabic (GA) was achieved by mutual titration of soy protein and gum arabic and was characterized using isothermal titration calorimetry (ITC), turbidity, sedimentation and ternary phase boundaries. In the first section, SP were titrated into GA (SP-to-GA titration) under salt-free condition (no added NaCl) at pH 3.0 and pH 5.6, respectively. ITC experiments displayed exothermic processes at both pH status, but the enthalpy changes (ΔH) at pH 3.0 was -0.70 ± 0.02 cal/g as compared to -0.10 ± 0.01 cal/g at pH 5.6. For SP-to-GA titration at pH 3.0, a sudden turbidity increase was observed at the critical SP/GA mass ratio (r_φ) of 0.42, which was approximately equal to the charge density ratio of GA and SP (0.36), indicating the charge compensation was achieved at phase separation point. In the second part, GA was titrated into SP (GA-to-SP titration) under salt-free condition at pH 3.0. An immediate turbidity increase was observed when GA was added into SP, while the sedimentation ratio measurement showed that the complex was unstable only in the GA/SP mass ratio range of 0.3–0.6. The ITC result showed a much higher ΔH than that for SP-to-GA titration. Effect of NaCl concentration on the complexing behavior by SP-to-GA titration at pH 3.0 was studied in the last. ΔHs and binding isotherms changed monotonically with the increase of salt concentration from 0 to 250 mM. However, turbidity measurement and phase boundaries revealed that the maximum phase separation was obtained at salt concentration of 100 mM.

1. Introduction

Complexing between oppositely charged proteins and polysaccharides is a ubiquitous colloidal phenomenon involved in the structuring of many biological systems. There has been considerable interest in protein-polysaccharide complexes in recent years, because of their potential applications in food industry as they are used in acid dairy drinks stabilization (Laurent & Boulenguer, 2003), emulsification (Dickinson, 2009; Guzey, Kim, & McClements, 2004; Guzey & Mcclements, 2007), foam stabilization (Miquelim, Lannes, & Mezzenga, 2010; Schmitt et al., 2005), fat replacement (Le Révérend, Norton, Cox, & Spyropoulos, 2010), encapsulation (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel; 2007), enzyme immobilization and recovery (Xia, Mattison, Romano, Dubin, & Muhoberac, 1997), and protein separation processes (Xu, Mazzawi, Chen, Sun, & Dubin, 2011).

Thermodynamic characterization is one of the most important issues in protein-polysaccharide complexing, from which a series of parameters can be obtained. Isothermal titration calorimetry (ITC) is widely used in the studies of proteins-polysaccharides, or in a broader sense, polyelectrolytes interactions (Aberkane, Jasniewski, Gaiani, Scher, & Sanchez, 2010; Du, Dubin, Hoagland, & Sun, 2014; Feng, Leduc, & Pelton, 2008; Xu et al., 2011). The complexing between oppositely charged protein and polysaccharide is generally found to be an exothermic process due to favorable electrostatic

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interactions (Aberkane et al., 2010; Girard, Turgeon, & Gauthier, 2003; Guzey & McClements, 2006; Schmitt et al., 2005; Sperber, Cohen Stuart, Schols, Voragen, & Norde, 2010; Vinayahan, Williams, & Phillips, 2010). In addition, to the favorable enthalpy changes, favorable entropic changes, such as the release of small counterions and water molecules, have also been reported to contribute to the protein-polysaccharide complexing (de Kruif, Weinbreck, & Vries, 2004; Sperber et al., 2010).

From the ITC data, the enthalpy change (ΔH) can be obtained directly and other thermodynamic parameters, such as the binding constant (K), and the binding number (n) can be achieved by fitting typically theoretical models. However, most of these models were based on systems of polysaccharide and low molecular weight proteins (Aberkane et al., 2010; Du et al., 2014; Hattori, Bat-Aldar, Kato, Bohidar, & Dubin, 2005; Vinayahan et al., 2010; Wang, Matei, Gronenborn, Ramstrom, & Yan, 2012; Xu et al., 2011). ITC studies of complexing behavior of protein-polysaccharides with similar molecular weight are rarely reported. Some authors (Feng et al., 2008) pointed out that colloidal polyelectrolyte complexes were actually kinetically frozen structures, for they were hardly a reversible, equilibrium processes. Therefore, binding constants and binding stoichiometries from thermodynamic models offered little more than curve fitting when considering complexes prepared from two high molecular weight polyelectrolytes (Feng et al., 2008). Yuan et al. (2013) reported the thermodynamic properties of glycinin/chitosan complexing at pH 3.5 and pH 4.5. They found an exothermic enthalpy change for the system at pH 4.5 due to the strong electrostatic interaction between glycinin and chitosan.

Soy proteins are composed of two proteins, 7S and 11S, with molecular weight of 180 kDa and 330 kDa respectively, which are much higher than those proteins commonly studied. Complexing of soy proteins-polysaccharide systems is attracting increasing interest recently. Several studies reported the associative interaction between soy proteins and polysaccharide (Huang, Sun, Xiao, & Yang, 2012; Jaramillo, Roberts, & Coupland, 2011; Liu et al., 2011; Murakami & Takashima, 2003). In the previous study, we found the complexing behavior of soy proteins/gum arabic mixtures as a function of pH (Dong et al., 2013). It was revealed that, with the increase of SP/GA ratio, critical pHs for the formation of insoluble complex shifted to higher values. At the same time, the pH value at which soy proteins and gum arabic carried the equal amount but opposite charges also changed in the same way. Therefore, our study indicated that charge compensation was fulfilled for SP/GA insoluble complex formation.

In this study, protein-polysaccharide complexes were studied by mutual titration of soy proteins and gum arabic at two pHs, at which SP and GA displayed strong or weak attraction interactions. Several complementary analytical techniques were used to characterize soy proteins-gum arabic interactions in aqueous solutions, and to study the nature of the complexes formed. Among them, isothermal titration calorimetry (ITC) measurements provided information about the enthalpy changes and stoichemistry of the interaction, while dynamic light scattering (DLS) and turbidity measurements (OD_{600}) yielded information about the formation of soluble and insoluble complex respectively. This work gave a more detailed understanding of the interactions between soy proteins and gum arabic and provided useful information in the development of functional protein ingredients through protein-polysaccharide complexing.

2. Materials and methods

2.1. Materials

Hexane defatted and flush desolventized soy flake, provided by Shandong Wonderful Industrial & Commercial Co. Ltd. (Dongying, China), had a protein content of 52.4% (dry basis) and nitrogen solubility index (NSI) of 85%.

Soy proteins were prepared as reported previously (Dong et al., 2013). The analysis showed that the dried powder had protein contents of 92.8% as determined by the Kjeldahl method (AOAC, 2000) with a nitrogen conversion factor of 6.25 on a dry basis.

Powder gum arabic (GA, Instant gum AA) was a gift from the Tianjin Jebsen Specialty Chemicals Co. Ltd. (Tianjin, China), and the composition was 2.2% protein, 10.7% moisture and 3.3% ash, and nearly lipid-free. Chemical analysis was conducted according to AOAC methods (2000): 979.09 for crude protein, 925.10 for moisture, and 923.03 for ash. Carbohydrate content was calculated on the basis of percent differential from 100%.

The average molar mass weight (Mw) of gum arabic, as determined by size exclusion chromatography (SEC-HPLC), was 327,000 g/mol. SEC-HPLC was performed with TSK-G 5000 column (Tosoh, Tokyo, Japan). The separation was carried out at 25 °C using 0.1 M $NaNO_3$ as eluant and a flow rate of 1.0 mL/min. All the other reagents were of analytical grade.

2.2. Preparation of SP and GA stock solutions

Soy proteins and gum arabic stock solutions were prepared by dispersing certain amount of biopolymer powder in double distilled water under gentle stirring at room temperature (25 ± 1 °C) for 2 h and left overnight at 4 °C to allow complete hydration of macromolecules. After adjusted to desired pH with 0.1 mol/L or 1 mol/L HCl, SP and GA solutions were centrifuged at 19,000 rpm (29,500 × g) for 30 min at 4 °C and the supernatants were filtered through 0.22 μm membrane. 0.02% (w/v) NaN_3 was added to inhibit bacteria growth and then the concentration of soy proteins was measured with the Kjeldahl method (AOAC, 2000). The gum arabic concentrations were assayed with the phenol-sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

2.3. Isothermal titration calorimetry (ITC)

Energetic and binding parameters from the complexing between soy proteins and gum arabic were investigated by ITC. For this experiment, dispersions were prepared in double distilled water. The SP and GA solutions were dialyzed extensively in double distilled water at pH 3.0 for 24 h, while only GA solution was dialyzed at pH 5.6 but no dialysis for SP solution because soy proteins tended to aggregate during the dialysis process. The dialysis water was preserved for ITC experiments and solution dilution. After that, the concentration of soy proteins was measured with the Kjeldahl method (AOAC, 2000), while the gum arabic concentrations were assayed with the phenol-sulfuric acid method (Dubois et al., 1956). Dialysis water was used as solvent to adjust the SP and GA solutions to desired concentrations (0.1% (w/v) and 1.0% (w/v) for SP and 0.1% (w/v) and 0.5% (w/v) for GA respectively). All solutions were degassed for 5 min at 20 °C prior to ITC measurements by means of a device provided with the ITC apparatus. The heat from buffer mismatching and dilute effect were measured by the blank titration of 1.0% (w/v) SP or 0.5% (w/v) GA solution into dialysis water, and the heat was subtracted from the raw data to obtain corrected enthalpy changes.

ITC experiments were performed with an iTC200 Microcalorimeter from MicroCal (GE Healthcare). 1.0% (w/v) SP solution (or 0.5% GA) was introduced in a 40 μL syringe, whereas 0.1% (w/v) GA solution (or 0.1% SP) was placed in the 200 μL measuring cell. The titration was performed at 25 °C by 19 successive injections (2 μL) of soy proteins with 90 s waiting time between each injection. The