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The effect of ionic strength on the rheology of pH-induced bovine serum albumin/κ-carrageenan coacervates

Changhoon Chai [a], Jooyoung Lee [b, c, *], Qingrong Huang [b]

[a] Department of Food and Nutrition, Kookmin University, Seoul 136-702, Republic of Korea
[b] Department of Food Science, Rutgers, The State University of New Jersey, 65 Dudley Rd., New Brunswick, NJ 08901, USA
[c] Engineering Division, Yonsei College for Freshman, Yonsei University, 85, Songdogwahak-ro, Yeonsu-gu, Incheon 406-840, Republic of Korea

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ABSTRACT

Protein/polysaccharide coacervates are frequently applied to food products to control the rheology. This study investigated the effect of ionic strength (I) on the rheology of pH-induced protein/polysaccharide coacervates. Bovine serum albumin (BSA) and κ-carrageenan were used as a model protein and a polysaccharide, respectively. As the formation of BSA/κ-carrageenan coacervates increased the turbidity of an aqueous mixture, pH_c, pH_ϕ, and pH_{max} values were identified corresponding to the pH of the formation of soluble coacervates, insoluble coacervates, and large insoluble coacervates respectively. Based on pH_c, pH_ϕ, and pH_{max}, a state diagram of BSA/κ-carrageenan coacervation versus pH and I was constructed. Involvement of salt in coacervation screened out the electrostatic interaction between BSA and κ-carrageenan coacervation, resulting in the shift of pH_c, pH_ϕ, and pH_{max} to lower pH. The shift was linearly changed to $1/I^{1/2}$ that corresponded to the Debye length. BSA/κ-carrageenan coacervates were more elastic than viscous. The transition from insoluble coacervates to large insoluble coacervates contributed to enhancement of the rheology, especially in elasticity. An increase in the I of a BSA/κ-carrageenan mixture reduced the degree of coacervation and the elasticity, and the viscosity of BSA/κ-carrageenan coacervates.

1. Introduction

Protein/polysaccharide coacervation is of continuing interest due to great flexibility in the engineering of mechanical and structural properties of foods, cosmetics, and pharmaceuticals. Previous studies of protein/polysaccharide coacervation achieved big advances in control of microscopic rheology, encapsulation of bioactive compounds, and design of drug delivery systems (Champagne & Fustier, 2007; Laneuville, Paquin, & Turgeon, 2005; Shimokawa, Saegusa, Wada, & Ishii, 2013).

Protein/polysaccharide coacervation involves separation of a protein/polysaccharide aqueous mixture into two immiscible phases, a dense phase concentrated with protein/polysaccharide coacervates and a diluted equilibrium phase arising mostly from electrostatic interaction between charged proteins and oppositely charged polysaccharides (Bungenberg de Jong, 1949). The electrostatic interaction liberates counterions and water molecules from the surface of proteins and polysaccharides which increases the entropy of the system and produces protein/polysaccharide coacervates (Ball et al., 2002; Ou & Muthukumar, 2006). Protein/polysaccharide coacervates are soluble or insoluble depending on their size and surface properties (Schmitt & Turgeon, 2011). The electrostatic interaction beyond the formation of soluble coacervates makes them insoluble and separated from a diluted phase (Schmitt & Turgeon, 2011).

The environmental parameters of a system, such as pH, ionic strength (I), and the protein to polysaccharide ratio, play critical roles in protein/polysaccharide coacervation (Bungenberg de Jong, 1949). Alteration of the surface charge of both the protein and the polysaccharide by a change in pH initiates and prolongs the ongoing of coacervation. Proteins and polysaccharides associate together at the critical pH (pH_c) where the protein and polysaccharide are oppositely charged, which forms soluble protein/polysaccharide coacervates (Xia, Dubin, Kim, Muhoberac, & Klimkowski, 1993). Coacervation at the pH of visual phase separation (pH_ϕ) beyond pH_c produces insoluble protein/polysaccharide coacervates, resulting in an abrupt increase in turbidity

* Corresponding author. Engineering Division, Yonsei College for Freshman, Yonsei University, 85, Songdogwahak-ro, Yeonsu-gu, Incheon 406-840, Republic of Korea. Tel.: +82 32 749 3165.
E-mail address: foodnano@yonsei.ac.kr (J. Lee).

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(Xia et al., 1993). Further coacervation increases the size, but decreases the number of protein/polysaccharide coacervates near the pH value of maximum turbidity (pH_{max}) (Kaibara, Okazaki, Bohidar, & Dubin, 2000). Both the *I* and the protein to polysaccharide ratio influence the pH_c, pH_ϕ, and pH_{max} values of coacervation. Addition of salt to the system suppresses coacervation due to a screening effect that prevents electrostatic interaction and reduces the pH_c, pH_ϕ, and pH_{max} values of protein/polysaccharide coacervation (Bungenberg de Jong, 1949; Weinbreck, de Vries, Schrooyen, & de Kruif, 2003). The higher the protein to polysaccharide ratio, the higher is the pH_ϕ value of protein/polysaccharide coacervation (Yang, Anvari, Pan, & Chung, 2012).

The effect of environmental parameters on the physicochemical properties of protein/polysaccharide coacervates is complicated, especially in application to foods having a wide range of both types and quantity of ingredients, such as salts and acids. In order for development and quality control of foods, the physicochemical characterization of protein/polysaccharide coacervates according to a combination of environmental parameters is important. In this study, the combination effect of pH and *I* on the rheological properties of model coacervates was investigated using bovine serum albumin (BSA), one of the simplest and the most characterized proteins (Jachimska & Pajor, 2012), and κ-carrageenan, one of the most characterized and widely used anionic polysaccharides (Piculell, 2006). The pH_c, pH_ϕ, and pH_{max} values of BSA/κ-carrageenan coacervation were obtained from turbidimetric titration of a BSA/κ-carrageenan aqueous mixture. A state diagram of BSA/κ-carrageenan coacervation was developed based on the pH_c, pH_ϕ, and pH_{max} values versus *I*. The values of the storage modulus (G') and the loss modulus (G'') representing the rheology of BSA/κ-carrageenan coacervates at each state were analyzed and compared in order to investigate the effect of *I* on the rheology of BSA/κ-carrageenan coacervates.

2. Materials and methods

2.1. Materials

BSA (lyophilized powder) and κ-carrageenan were purchased from Sigma-Aldrich Co. (USA). A standard sodium hydroxide solution (0.05 mol/L), a standard hydrogen chloride solution (0.05 mol/L), and analytical grade sodium chloride were purchased from Fisher Scientific (USA). Deionized water was used for all solutions.

2.2. Preparation of BSA/κ-carrageenan aqueous mixtures

BSA and κ-carrageenan were dispersed separately in 0.01, 0.05, 0.1, 0.3, and 0.5 mol/mL sodium chloride solutions to prepare stock solutions. BSA and κ-carrageenan in sodium chloride solutions were mixed to prepare BSA/κ-carrageenan aqueous mixtures. BSA to κ-carrageenan ratios were 2:1, 5:1, 10:1, and 30:1. The total concentration of macromolecules (sum of BSA and κ-carrageenan) in the mixtures was 5 g/L. The mixtures were adjusted to pH 6.5 (±0.05). All solutions were filtered through a 0.45 μm filter (Millipore Ltd. USA) prior to use.

2.3. Turbidimetric titrations

Turbidimetric titrations were carried out in a sequence of titration, pH measurement, and turbidity measurement, and repeated until apparent visual separation of the BSA/κ-carrageenan aqueous mixture into two phases. A few drops of a 0.05 mol/L hydrogen chloride solution was added to the mixture, which was then stirred gently for 2–10 min until the pH was equilibrated. pH and turbidity values of the mixture were then measured. A colorimeter (PC 910 colorimeter, Brinkmann Instruments, USA) equipped with a 420 nm filter was used for turbidity measurements. Turbidity (τ) was defined as $\tau = 100 - (T/T_{DI\ water}) \times 100$, where *T* is the value of light transmittance through a solution obtained using a colorimeter. During the turbidimetric titration, the temperature of the solution was maintained at 25 °C.

2.4. Rheological measurements

A BSA/κ-carrageenan aqueous mixture in differently concentrated sodium chloride solutions with a 10:1 BSA to κ-carrageenan ratio was adjusted to pH 4.7 using 0.05 mol/L hydrogen chloride, and centrifuged at 6000 × *g* for 30 min. BSA/κ-carrageenan coacervates were collected from the centrifuged precipitate. Dynamic rheological properties of collected coacervates were analyzed using a strain-controlled rheometer (Advanced Rheometric Expansion System, TA Instruments, USA) with parallel plate geometries (25 or 50 mm in diameter). The operational temperature of the rheometer was set at 25 °C. BSA/κ-carrageenan coacervates were loaded and maintained on the plate for 10 min for thermal equilibrium of the coacervates. The storage modulus (G') and loss modulus (G'') values were measured while the frequency was varied from 0.1 to 100 rad/sec.

3. Results and discussion

3.1. pH-induced BSA/κ-carrageenan coacervation

The BSA surface charge depends upon the charge state of the amino acid side chain on the protein surface. As the amino acid side chain has its own pK_a, its charge state is modulated by the environmental pH. BSA can participate in electrostatic interactions with κ-carrageenan if $pH < pK_a$ of the amino acid side chain on the BSA surface (Tanford & Kirkwood, 1957; Tanford, Swanson, & Shore, 1955).

The association between BSA and κ-carrageenan at pH_c that is enhanced by reduction of the electrostatic repulsion and the domination of the cation on the BSA surface, produces soluble BSA/κ-carrageenan coacervates with a slight increase in turbidity (Fig. 1). The turbidity of the BSA/κ-carrageenan coacervates abruptly increased at pH_ϕ (Fig. 1) with an increase in the number/size of BSA/κ-carrageenan coacervates. Turbidity reached a

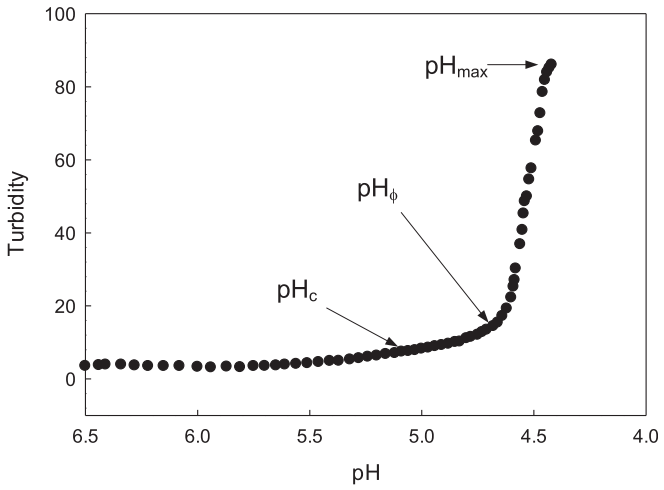

Fig. 1. Turbidities of BSA/κ-carrageenan aqueous mixtures (BSA: κ-carrageenan = 10:1) in a 0.1 mol/L sodium chloride solution after titration with 0.05 mol/L hydrogen chloride.