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Unique characteristics of self-assembly of bovine serum albumin and fucoidan, an anionic sulfated polysaccharide, under various aqueous environments

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ABSTRACT

Interpolymeric complexes of bovine serum albumin (BSA)/fucoidan (weight ratio of 5:1) were prepared by decreasing pH in the absence and presence of NaCl. The turbidity drastically increased at pH 4.5 ($pH_{\varphi 1}$) and the maximum biopolymer interactions induced at pH 4.0 (pH_{opt}). The viscosity of BSA/fucoidan complexes at pH 4.0 was 1.5 times higher than that of individual protein solutions. These results reflected that both BSA and sulfated dominant fucoidans (NH_3^+ and $-SO_3^-$) favored the formation of BSA/fucoidan complexes with very dense and compact internal structures. On the other hand, increasing NaCl concentration (from 0 to 1 M) shifted the critical pH ($pH_{\varphi 1}$) to a more acidic value, which may be explained by salt screening. Interestingly, 0.01 M of NaCl dissociated the aggregated BSA/fucoidan complexes into a soluble state at pH = 4.5.

1. Introduction

Proteins and polysaccharides are the two of the most significant natural macromolecules, and their interactions are widely used in a variety of food systems to form stable foams or multi-layers emulsions (Gu, Decker, & McClements, 2007; Jourdain, Leser, Schmitt, Michel, & Dickinson, 2008; Sánchez & Patino, 2005; Semenova, Belyakova, Polikarpov, Antipova, & Dickinson, 2009), encapsulation processes (Dong et al., 2011; Matalanis, Jones, & McClements, 2011; Nori et al., 2011) and the formulation of new structures and textures (Corredig, Sharafbafi, & Kristo, 2011; Frith, 2010; Lizarraga, Vicin, González, Rubiolo, & Santiago, 2006; Mandala, Michon, & Launay, 2004). Protein/polysaccharide interactions may undergo either of two different types of phase behavior: segregative or associative phase separation (Doublier, Garnier, Renard, & Sanchez, 2000; Kasapis, Norton, & Ubbink, 2009, chap. 11; Patino & Pilosof, 2011). The latter phenomena give rise to the formation of complex coacervates, co-precipitates (interpolymeric complexes), gels, and soluble complexes (Turgeon, Beaulieu, Schmitt, & Sanchez, 2003). Until now, a large number of reviews have been published on the behaviors of solutions with protein/polysaccharide interactions (de Kruif, Weinbreck, & de Vries, 2004; Schmitt & Turgeon, 2011; Turgeon, Schmitt, & Sanchez, 2007; Veis, 2011) and there have been numerous original papers published on the formation of biopolymer complexes as a function of various parameters (Bastos et al., 2010; Bengoechea, Jones, Guerrero, & McClements, 2011; Elmer, Karaca, Low, & Nickerson, 2011; Fioramonti, Perez, Aríngoli, Rubiolo, & Santiago, 2014; Giancone, Torrieri, Masi, & Michon, 2009; Laneuville, Paquin, & Turgeon, 2000; Lee & Hong, 2009; Souza, Rojas, Melo, Gaspar, & Lins, 2013; Weinbreck, de Vries, Schrooyen, & de Kruif, 2003; Weinbreck, Nieuwenhuijse, Robijn, & de Kruif, 2003; Weinbreck, Nieuwenhuijse, Robijn, & de Kruif, 2004; Weinbreck, Tromp, & de Kruif, 2004). According to precedent studies, the driving force for these non-covalent interactions are the electrostatic interactions between oppositely charged biopolymers, which can be affected by both extrinsic factors (protein/polysaccharide ratio, pH, ionic strength, and temperature) and intrinsic factors (molecular weight, molecular structure, net charge and flexibility of chains).

de Kruif et al. (2004) reported an overview of protein–polysaccharide interactions categorized by the charge densities of polysaccharides. According to this review, strong sulfate (κ-carrageenan, ι-carrageenan, dextran sulfate) and strong phosphate polysaccharides (sodium hexametaphosphate, exopolysaccharide B40) tend to form precipitates with proteins rather than with liquid coacervate phases. Weinbreck, de Vries, et al. (2003), Weinbreck,

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Nieuwenhuijse, et al. (2003), and Weinbreck, Nieuwenhuijse, et al. (2004) described the influence of the charge density of a polysaccharide on the formation of whey protein isolate (WP) with different types of polysaccharides: carboxylated (gum arabic), sulfated (λ-carrageenan), and phosphated (EPS B40) polysaccharide. For the WP/arabic gum (carboxylated) system, the mixture formed a complex coacervate driven by the interaction of β-lg and arabic gum for pH between 2.5 and 4.8, where both biopolymers are oppositely charged. For WP/λ-carrageenan (strong sulfated polysaccharide), complexes appeared to form precipitates, and for WP/EPS B40 (a highly phosphated polysaccharide), there was intermediate behavior between that of acacia gum and that of λ-carrageenan. This suggests that the molecular attraction of $-NH_3^+$ groups on proteins for $-OSO_3^-$ groups on sulfated polysaccharides was much stronger than that for $-CO_2^-$ groups on carboxylated polysaccharides.

By contrast, Girard, Turgeon, and Gauthier (2002) reported the amount of interbiopolymer complexes in β-lg/low-methylated (LM) and β-lg/high-methylated (HM) pectin systems under the same conditions (β-lg/pectin ratio of 4:1, pH 4.5). In their work, the proportion of β-lg/LM pectin complexes (96%) was greater than that of β-lg/HM pectin (78%), and this difference can be attributed to differences in polysaccharide conformations and linear charge densities between the two pectin types. Furthermore, as the carboxyl groups on the polysaccharide chains favored the formation of hydrogen bonds, the extent of electrostatic attraction was dependent on the solution condition. LM pectin forms complexes with β-lg, even at high ionic strengths, due to its higher local charge density (Sperber, Cohen Stuart, Schols, Voragen, & Norde, 2009).

BSA is a model globular protein with a molecular weight of about 66.5 kDa, an amphiphilic structure, and a flexible conformation. Recently, Ru, Wang, Lee, Ding, and Huang (2012) reported that a mixture of BSA and pectin forms tighter coacervate networks at various salt concentrations and initial protein/polysaccharide ratios. Separately, Jamróz et al. (2014) reported that BSA interacted with furcellaran at pH = 4 to form a core complex for further layer-by-layer encapsulation.

Fucoidan is an anionic polysaccharide that is extracted from brown seaweed, such as kombu and wakame. Fucoidan contains considerable amounts of L-fucose and sulfated groups, along with lesser amounts of D-xylose, D-mannose, and glucuronic acid (Gideon & Rengasamy, 2008). The composition and structure of fucoidan varies depending on the algal source considered, but the main framework usually consists of α-1,3-linked-L-fucose-4-sulfate; a repeating sequence of alternating α(1 → 3) and α(1 → 4) glycosidic bonds is also possible (Berteau & Mulloy, 2003; Kim et al., 2008). Moreover, the structure of the fucoidan molecule has a formal resemblance to that of the fucosylated chondroitin sulfate from the body walls of sea cucumbers (Duarate, Cardoso, & Noseda, 2001). It is commonly used in bioactive materials because of the high density of sulfated groups attached to it (Chevolot et al., 1999), and electrostatic combination of fucoidan with BSA enhances the stability of an oil-in-water emulsion (Kim & Shin, 2009; Kim, Shin, & Hong, 2010).

To our knowledge, complexation of BSA with ι-carrageenan is characterized by a stronger electrostatic interaction than complexation with κ-carrageenan. Furthermore, complexation of BSA with dextran sulfate features an even stronger electrostatic interaction than that with ι-carrageenan. These interactions are dependent on the charge densities of natural sulfated polysaccharides (Galazka, Smith, Ledward, & Dickinson, 1999). However, BSA/fucoidan (strong sulfated polysaccharide) complexes have not been analyzed before. Therefore, the objective of this research is to understand the physicochemical properties of self-assembled complexes between BSA and fucoidan as they are affected by various factors, to study the nature of polysaccharide (charge density, molecular structure, and conformation), and to propose the application of their functional properties in the food industry.

2. Materials and methods

2.1. Materials

Lyophilized bovine serum albumin (Fraction V, minimum 96% by agarose gel electrophoresis, product #A4503) was purchased from Sigma Chemical Co. and fucoidan extracted from *Undaria pinnatifida* was obtained from Haewon Biotech, Inc (Seoul, Korea). In contrast to common fucoidans, this polysaccharide consists mainly of both β-D-galactopyranose and α-L-fucopyranose at a ratio of 1:1.1 with xylose and mannose as minor sugars as shown in Fig. 1 (Kim & Shin, 2009; Synytsya et al., 2010). The infrared spectrum of the fucoidan purified *U. pinnatifida* reveals strong absorption peaks at approximately 1630 cm^{-1} reported by Liu et al. (2012) and at 1256 cm^{-1} by Synytsya et al. (2010). These bands represent typical sulfated residues and provide evidence that fucoidan is mostly composed of highly sulfated galactofucan. The weight-averaged molecular weight (M_w) and the radius of gyration (R_g) of fucoidan were 6.56×10^5 (g/mol) and 48.43 ± 0.02 (nm), respectively as determined using multi-angle laser light scattering analysis (BI-9000AT, Brookhaven Instruments Co., New York, USA) equipped with a He–Ne laser (632.8 nm) as a light source (data not shown). Secondly, the second virial coefficient was 2.03×10^{-5} ($A_2 > 0$), indicating that the polymer appeared to interact with solvent molecules and other polymer molecules, that is, the polymer particles tend to remain in a stable solution. The d*n*/d*c* value measured by the differential refractometer was 0.1292. This value was similar to that reported by Rioux, Turgeon, and Beaulieu (2007). Fucoidan behaved as a branched polysaccharide, with many dangling sulfate chains located at the surface, which were highly soluble, and fucoidan had a low viscosity in water at room temperature.

In this experiment, analytical grade reagents and deionized water were used for the preparation of all solutions. All measurements were performed in triplicate on freshly prepared samples, and they are reported as means ± standard deviations.

2.2. Preparation of BSA/fucoidan mixture

Biopolymer stock solutions were prepared by dissolving BSA (5%, w/w) and fucoidan (1%, w/w) in 100 mM phosphate buffer (pH 7.0) until the powders were dispersed completely. BSA/fucoidan mixtures containing BSA (0.5%, w/w) and fucoidan (0.1%, w/w) were prepared and the pH (2.5–8.0) was adjusted using 1 M HCl or NaOH solutions. Ionic strength (0–1 M) was controlled using NaCl. In the preliminary work, the optimum ratio of BSA to fucoidan (Pr:Ps) to form an insoluble complex was investigated at different biopolymer mixing ratios ranging from 1:1 to 25:1. A maximum optical turbidity was observed at a ratio of 5:1, and this ratio was selected as suitable for the formation of protein/polysaccharide biopolymer complexes.

2.3. Characterization of BSA/fucoidan mixture

2.3.1. Turbidity measurements

The turbidities of biopolymer mixtures at different pH and ionic strengths were measured at 514 nm using a UV–visible spectrophotometer (Amersham Biosciences, Uppsala, Sweden). Sample solutions were vortexed for 5 s prior to measurements, and distilled water was used as a blank control. All samples were run in triplicate and the results are reported as the mean and standard deviation.