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Heteroprotein complex formation of bovine serum albumin and lysozyme: Structure and thermal stability

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ABSTRACT

The formation of a heteroprotein complex obtained by the interaction of bovine serum albumin (BSA) and lysozyme (Lys) was investigated by pH variation using turbidimetric analysis and zeta potential (ζ) at different protein ratios and NaCl concentrations. The complexes were formed in a pH range between 8.0 and 11.0, with the ratio r = 0.5 at pH 9.0 presenting the highest complexation. The addition of NaCl decreased the interaction at concentrations of 10 mM. The complex formation occurred between the isoelectric points (pI) of the proteins, close to a balance of charges, mainly by electrostatic interactions with some participation of hydrogen bonds. Differential scanning calorimetry suggested that the interaction gave rise to a new biopolymer due to the formation of a single denaturation point at 67 °C. The structures formed had an average size of ~1.7 μm, well above that of the isolated proteins, and microscopic analysis revealed that the complexes had a globular structure. BSA/Lys complexes may be a potential bioactive encapsulating agent and may be used as a food ingredient.

1. Introduction

Coacervation is defined as the colloidal separation of two-phase liquid systems (IUPAC, 1997), and complex coacervation, known as an associative phase separation, is motivated by the attraction of oppositely charged biopolymers (Souza & Garcia-Rojas, 2017; Tolstoguzov, 1991; Turgeon, Schmitt, & Sanchez, 2007; Zhang, Zhang, Abbas, & Karangwa, 2013). Coacervate complexes are formed mainly by electrostatic interactions, and for this reason, their formation has been influenced by variations in pH and ionic strength because they can alter the surface charge density of the molecules (Li, Shim, Wang & Reaney, 2012). Four pH values can be identified during the formation of complexes: pH_c represents the formation of soluble complexes between biopolymers and can be observed with a slight increase in turbidity during the titration; $pH_{\theta 1}$ marks the formation of insoluble complexes (coacervates) and is observed as the rapid increase in turbidity; pH_{max} marks the maximum point of turbidity and represents the point of electrical equivalence; and $pH_{\theta 2}$ occurs shortly after reaching the maximum and is observed as the reduction in turbidity, indicating the end of the complex formation (Kruif & Tuinier, 2001; Liu, Shim, Wang, & Reaney, 2015; Souza & Garcia-Rojas, 2017; Turgeon, Beaulieu, Schmitt, & Sanchez, 2003).

A number of studies have reported the formation and potential technological application of the coacervate complexes between polysaccharides and proteins (Souza & Garcia-Rojas, 2015; Water et al., 2014; Yuan, Kong, Sun, Zeng, & Yang, 2017). More recently, studies have been proposed to understand protein-protein complex coacervation (Anema & de Kruif, 2014; Anema & Kruif, 2013; Pathak, Rawat, Aswal, & Bohidar, 2016). A heteroprotein complex between two animal proteins, lactoferrin and β-lactoglobulin, has been well studied (Yan et al., 2013; Flanagan et al., 2015). However, Adal et al. (2017) recently proposed a study of heteroprotein complexes between lactoferrin and pea proteins. Despite a limited number of studies, these heteroprotein complexes have already been shown to be a versatile agent for the protection and transport

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of bioactive foods, as reported by Diarrassouba et al. (2015), when proposing the encapsulation of vitamin D3 using β-lactoglobulin and lysozyme, and Chapeau et al. (2016), when using lactoferrin and β-lactoglobulin for the encapsulation of vitamin B9.

Among food ingredients, proteins are one of the most indispensable due to their functional and technological properties (Howell, Yeboah & Lewis, 1995). Eggs, especially egg whites, are one of the most consumed high-protein foods with high nutritional and functional value (Kovacs-Nolan, Phillips, & Mine, 2005; Stadelman & Cotterill, 1990). Lysozyme is a globular glycoprotein with enzymatic action presenting in high concentrations in egg whites (3.4%). It has a molar mass of 14 kDa, an isoelectric point (pI) of 10.7 and numerous functional properties, which are mainly antimicrobial (Sgarbieri, 1996).

Whey, mainly obtained from cheese production, is another source rich in proteins of high nutritional value but has been mostly discarded in small dairy properties (Pelegrine & Carrasqueira, 2008). Bovine serum albumin is one of the protein constituents of whey, consisting of approximately 583 amino acid residues, with a molar mass of 66 kDa and a pI of approximately 5.0. It is one of the most studied proteins of this group, particularly because of its structural similarity with human serum albumin (HSA) (Zhao, Li, Carvajal, & Harris, 2009).

The objective of this work was to study the influence of pH, NaCl concentration, and total mass ratio of proteins in the process of heteroprotein complex formation resulting from the interaction between bovine serum albumin and lysozyme and to characterize these complexes by Fourier Transform Infrared Spectroscopy (FTIR), microscopy (SEM and Optical) and Differential Scanning Calorimetry (DSC).

2. Materials and methods

2.1. Materials

Lysozyme (Lys, purity ≥ 90%) and bovine serum albumin (BSA, Purity ≥ 96%) were obtained from Sigma-Aldrich (St. Louis, USA). Sodium chloride (NaCl, purity> 99%), hydrochloric acid (HCl), and sodium hydroxide (NaOH) were purchased from VETEC® Ltd, (Rio de Janeiro, Brazil). Ultrapure water with a conductivity of 0.05 μS/cm was used (Gehaka, Master-P & D, Brazil).

2.2. Formation of complexes

2.2.1. Sample preparation

The concentrations of Lys and BSA were set at 0.1% w/w in the system. BSA and Lys solutions were used as controls and seven ratios (r) of BSA:Lys were studied: 8 (8:1); 4 (4:1); 2 (2:1); 1 (1:1); 0.5 (1:2); 0.25 (1:4); and 0.125 (1:8). To evaluate the influence of NaCl on the complex formation, five different NaCl concentrations (0; 10, 50, 100 and 300 mM) were studied for the BSA/Lys ratio, which resulted in greater turbidity. For the preparation of the solutions, the proteins were weighed using an analytical balance (Shimadzu, AY 220, Philippines) and agitated with a magnetic stirrer (Novatecnica, model NT101, Brazil) for 30 min. The pH of the solutions was pre-adjusted to 2.0 and measured using a benchtop pH meter (mPA-210, Tecnopon, Brazil) for subsequent turbidimetric titration.

2.2.2. Turbidimetric titration

The sample transmittance was measured at a wavelength of 400 nm in a 1-cm quartz cuvette using a spectrophotometer (Biochrom mod. Libra S12, England). The equipment was calibrated for 100% transmittance (T) with ultrapure water. The turbidity was defined as τ (cm^{-1}), given by Equation (1), where I is the incident light intensity and I_0 is the light intensity after traversing the sample (Gulão, de Souza, Andrade & Garcia-Rojas, 2016). Solutions containing a defined BSA/Lys ratio and the respective NaCl concentration had their pH adjusted (2.0–12.0) with HCl and NaOH with the aid of a bench pH meter and a magnetic stirrer. The pH of the solutions was monitored, and a 1-mL aliquot was sampled to measure the transmittance value. All titrations were performed at room temperature (~25 °C) with four replicates, and the interval between measurements was approximately 1 min (Adapted from Gulão, Souza, Silva, Coimbra, & Garcia-Rojas, 2014).

$$\tau = -ln\,(I/I_0) \quad (1)$$

2.3. ζ- potential

The ζ- potential of the isolated proteins and the ratios studied were determined by Zetasizer (Malvern Instruments, Nano-ZS, UK). Samples (10 mL) at 0.1% (w/w) were placed on the titrater (Malvern Instruments, MPT-2, UK). The pH of the solutions were adjusted with the aid of 500 mM HCl, 250 mM NaOH, and 25 mM NaOH, and the analyses were conducted as a function of pH (2.0–12.0) in the range of 0.25 ± 0.1. The ζ- potential was calculated based on the mathematical model of Smoluschwsky, and each sample was measured in triplicate at 25 °C.

2.4. Particle size

The hydrodynamic diameter (d.nm) of the BSA/Lys complexes, in addition to the BSA and Lys standard solutions, were determined at a fixed pH by the DLS (Dynamic Light Scatering) technique. This was done using a Zetasizer (Malvern Instruments, Nano-ZS, UK) equipped with a He-Ne laser at a wavelength of 632.8 nm and a fixed detection angle of 90°. Samples were placed in glass cuvettes and measured in triplicate at 25 °C.

2.5. Characterization of heteroprotein complexes

For the characterization, was chosen the complexes in the ratio, pH and NaCl concentration according to the item 2.2.2. After preparation, the solution had its pH adjusted and was allowed to stand at 4 °C overnight. Then, the solution was centrifuged (Ortoalresa, digest 21 R, Spain) at 500×g for 10 min at a temperature of 20 °C (Anema & de Kruif, 2014). The supernatant was then discarded and the precipitate was placed in the ultra-freezer (Terroni, COLD 120, Brazil) at −40 °C for approximately 24 h. Soon after, it was placed in the benchtop freeze dryer (Terroni, Enterprise I, Brazil) for drying and stored in a desiccator with silica gel until use.

2.5.1. Fourier Transform Infrared Spectroscopy (FTIR)

Infrared spectra were obtained for the BSA and Lys standard samples and the lyophilized complexes of the defined BSA/Lys ratios. The analyses were performed with a FTIR spectrometer (Bruker, Vertex 70, Germany) using KBr (potassium bromide) tablets and were read in the range of 4000–500 cm^{-1}.

2.5.2. Optical microscopy

An aliquot of the coacervate complex in 0.1% (w/w) solution, r = 0.5, and pH 9.0, was placed between a lamina and coverslip, transported to the optical microscope Axioplan (Zeiss, Gottingen, Germany) coupled to an AxioCam MRc camera (Zeiss, Gottingen, Germany) and viewed at 100× with oil immersion.