International Journal of Biological Macromolecules 109 (2018) 467–475

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Interpolymer complexation of egg white proteins and carrageenan: Phase behavior, thermodynamics and rheological properties

Clitor J.F. Souza[a], Clyselen S.F. Souza[b], Lívia P. Heckert Bastos[a], Edwin E. Garcia-Rojas[a,b,*]

[a] Programa de Pós-graduação em Ciência e Tecnologia de Alimentos (PPGCTA), Universidade Federal Rural de Rio de Janeiro (UFRRJ), Rodovia BR 465, Km 7, Seropédica/RJ, 23890-000, Brazil
[b] Laboratório de Engenharia e Tecnologia Agroindustrial (LETA), Universidade Federal Fluminense (UFF), Av. dos Trabalhadores, 420, Volta Redonda/RJ, 27255-125, Brazil

ARTICLE INFO

Article history:
Received 25 October 2017
Received in revised form
19 December 2017
Accepted 20 December 2017
Available online 21 December 2017

Keywords:
Ovalbumin
Lysozyme
Polysaccharide
Complexation
Biopolymers

ABSTRACT

The complexation between lysozyme/carrageenan and ovalbumin/carrageenan was studied *in situ* using acidification. The complexes were analyzed in solutions with different NaCl concentrations and different protein/polysaccharide ratios. As the protein/polysaccharide ratio increased from 1:1 to 10:1, critical structure forming events (i.e., those associated with soluble, insoluble and large insoluble complexes) shifted to higher pH values for ovalbumin/carrageenan followed by decrease of G' values at ratios of 5:1 and 10:1. The increase in the ratio of lysozyme/carrageenan complexes suppressed the critical pH transition points that led to the formation of large insoluble complexes from pH 12.0 until 1.0, and the values of G' increased simultaneously, reaching the highest value at a ratio of 10:1. Addition of salt to the ovalbumin/carrageenan and lysozyme/carrageenan mixtures suppressed the electrostatic interaction between proteins and carrageenan at lower pH values and the critical pH transitions points, whereas at a ratio of 3:1 with a 0.01 M concentration, the coacervate yield of the complex reached 79.6% ± 0.6 and 93.7% ± 4.8 for the ovalbumin and lysozyme complexes, respectively. The rheological data associated with microscopy images show that interpolymer complexes with heterogeneous structures were formed for both complexes, and we suggest that complexes have a great potential to improve or extend the texture, mechanical stability, consistency, and taste of food products.

1. Introduction

Protein/polysaccharide coacervation is of continuing interest due to its great flexibility in the engineering of mechanical and structural properties of foods, cosmetics, and pharmaceuticals [1]. In foods, proteins and polysaccharides are the most important structure-forming ingredients and their use in mixed systems can improve or extend their texture, mechanical stability, consistency, and ultimately, appearance and taste [2–4].

Mixing a protein with a polysaccharide into an aqueous solution may generate one of several situations depending on polymer-polymer and solvent-polymer attractive or repulsive interactions. A greater understanding of the factors that affect biopolymer interactions is required, particularly for those of electrostatic origin [5]. Attractive interactions between two biopolymers can become evident in various ways: (i) the formation of a small soluble complex, manifesting itself in murky solutions, (ii) the formation of a homogeneous weak gel, if interactions are weak, and (iii) the precipitation of both biopolymers, if interactions are strong [6]. Therefore, a good understanding of the interactions between proteins and polysaccharides is vital for the design and development of new functional foods, and has become a topic of investigation

The environmental parameters of a system, such as pH, ionic strength, the protein to polysaccharide ratio, the amount of total solids, the rate of acidification, and the shear rate during acidification play critical roles in protein/polysaccharide coacervation [1,7,8]. Alteration of the surface charge of both the protein and the polysaccharide by a change in pH initiates coacervation. The critical pH (pHc) occurs where the protein and polysaccharide are oppositely charged, which forms soluble protein/polysaccharide coacervates [9]. Coacervation at the pH of visual phase separation (pHφ) beyond pHc produces insoluble protein/polysaccharide coacervates, resulting in an abrupt increase in turbidity [10]. Further coacervation increases the size, but decreases the number of

* Corresponding author.
E-mail address: edwin@vm.uff.br (E.E. Garcia-Rojas).

https://doi.org/10.1016/j.ijbiomac.2017.12.116
0141-8130/

protein/polysaccharide coacervates near the pH value of maximum turbidity (pHmax) [11].

Coacervates or polysaccharide-protein complexes are attractive to industry because they are natural products that are obtained from commonly used ingredients and are therefore inexpensive and easy to regulate and approve for use in food [12]. Ovalbumin is the major egg white protein that is synthesized in the hen's oviduct and accounts for 54% of all egg white proteins [13]. The molecular weight of ovalbumin is 45 kDa with 386 residues of amino acids. Ovalbumin does not have a classical N-terminal ladder sequence [14], but has three sites of post-synthetic modification in addition to the N-terminal acetyl group. The amino acid composition of ovalbumin is unique compared to other proteins, and for this reason, ovalbumin has many functional properties such as antimutagenic, anticarcinogenic [15], immunomodulatory [16] and antioxidant [17] properties. Ovalbumin has been used extensively in food technology because of its emulsifying and stabilizing properties. Lysozyme is another important protein that is found in egg white. The molecular weight of lysozyme is 14.4 kDa, and it consists of a single polypeptide chain with 129 residues of amino acids. In nature, this protein is found as a monomer but it is occasionally present as a dimer with more thermal stability. It is considered to be a strong basic protein present in egg white [18]. Lysozyme has 4 disulfide bridges that lead to high thermal stability, and its isoelectric point is 10.7. In the food industry, lysozyme is one of the major bacteriolytic proteins that has the capability of controlling foodborne pathogens such as *Listeria monocytogens* and *Clostridium botulinum* [19,20], which are considered to be the two main pathogens that cause problems in the food industry.

Carrageenan is a polymer that can be obtained from several genera and species of marine algae class Rodophyta [21]. Carrageenan is a sulfated polygalactan that contains 15–40% of ester-sulfate and has an average relative molecular mass that is much greater than 100 kDa. It is composed of alternating units of D-galactose and 3,6-anhydro-galactose (3,6-AG) joined by α-1,3 and β-1,4-glycosidic linkage. Carrageenan is classified into various types such as λ, κ, ι, ε, and μ, all of which contain 22–35% sulfate groups [22]. Carrageenan has no nutritional value; however, it is used in food preparation for its gelling, thickening, and emulsifying properties [23].

The present study aims to elucidate the influence of protein, pH, ionic strength and the ratio of protein/polysaccharide in the formation of complexes between egg white proteins and carrageenan. Complexation was investigated using the turbidimetric method, isothermal titration calorimetry and a dynamic rheometer.

2. Materials and methods

2.1. Materials

Lysozyme (Lyso purity >90%), ovalbumin (Ova; purity >90%) and carrageenan (Ca) were obtained from Sigma Chemicals (St. Louis, USA). Sodium chloride (NaCl, purity >99%), hydrochloric acid (HCl 0.5 M), and sodium hydroxide (NaOH 0.5 M) were purchased from VETEC® Ltda, (Rio de Janeiro, Brazil). The water that was used was ultrapure with a conductivity of 0.05 μS/cm (Gehaka-Master P&D - Brazil). Stock solutions of carrageenan (0.1% w/w), ovalbumin (0.1-1% w/w) and lysozyme (0.1-1% w/w) were prepared by gently stirring the powders in water for 24 h at room temperature (25 °C).

2.2. Formation of complexes

2.2.1. Preparation of complexes

The concentration of carrageenan that was used was 0.1% w/w and the concentration of lysozyme or ovalbumin that was used varied from 0.1-1% w/w. Five ratios of protein:Ca (1:1, 2:1, 3:1, 5:1, 10:1) were evaluated. To determine the effect of NaCl on complex formation, Lyso:Ca or Ova:Ca complexes were formed in five concentrations of NaCl (0.01, 0.05, 0.1, 0.2, 0.4 M). The protein:Ca mixtures were stirred and adjusted to pH 12.0 prior to turbidimetric measurements.

2.2.2. Turbidimetric measurements

pH-dependent turbidity was measured at a wavelength of 600 nm using a spectrophotometer (Biochrom mod. LIBRA S12, England) that was calibrated with ultrapure water to 100% transmittance (%T). Turbidity was defined as 100 −%T. With the aid of a magnetic stirrer (Nova Tecnica, NT 101, Brazil) and a pH meter (Tecnopon, mPA-210, Brazil) the pH levels of the solutions were adjusted (12.0–1.0) with HCl (0.5 M). Measurements of complexes and the solutions with biopolymer isolates were made at room temperature (25 °C), and each sample was measured four times at 1 min intervals.

2.2.3. Complex coacervate yield

The data that were obtained from the turbidimetric titration were used to choose the region (pH_{max}) with the highest value of turbidity for different ratios of protein/carrageenan and their respective concentrations of NaCl. The complexes were formed 72 h after the samples were centrifuged at a temperature of 5 °C (Olto Alresa, Digicen 21R, Spain) at 4226*g* for 30 min. The supernatant was removed, and the precipitate was frozen and lyophilized (Terroni mod. Enter 1B, Brazil). The complex coacervate yield (CCY) was determined using the following equation [24].

$$CCY\ \ (\%) = \frac{Weight\ \ of\ complexes}{total\ weight\ of\ \ (protein + polyssacharide + NaCl)} x100$$

All analyses were performed with three independent samples, and a simple analysis of variance was performed using the Tukey test to determine the difference between the means ($p < 0.05$).

2.3. Zeta- potential

A Zetasizer Nano ZS90 (Malvern Instruments, Worcestershire, UK) was used to determine zeta (ζ)-potentials. Lysozyme, ovalbumin and carrageenan stock solutions and Lyso:Ca or Ova:Ca complexes that formed in 0.01 M NaCl were diluted to 0.01% w/w and were transferred to an MPT-2 autotitrator (Malvern Instruments, Worcestershire, UK) that adjusted pH using 0.5 M NaOH, 0.25 M HCl and 0.025 M HCl solutions. pH was varied from 12.0 to 1.0 by 0.5 unit increments with a confidence interval of ± 0.1 unit. ζ-potentials were calculated using the Smoluschwsky mathematical model. Each experiment was performed three times and sample readings were made in triplicate at 25 °C.

2.4. Isothermal titration calorimetry (ITC)

ITC experiments were performed using a Nano-ITC system (TA Instruments, New Castle, DE, USA). Solutions of lysozyme (0.6 mM) and carrageenan (0.005 mM) were prepared in a 10 mM citrate buffer at pH 5.0. And solutions of ovalbumin (0.6 mM) and carrageenan (0.003 mM) were prepared in a 10 mM citrate buffer at pH 3.0;

Both protein and carrageenan solutions were stirred at room temperature until they were fully dissolved. The solutions were then stored at 4 °C for 24 h to ensure complete hydration and equilibration of the biopolymers. The lysozyme, ovalbumin and carrageenan solutions were dialyzed using a 3.5-kDa-cutoff dialysis tube (Sigma-Aldrich, Midi 3500, USA) for 24 h to ensure pH and ionic balance. The dialysis buffer was replaced six times. The lysozyme, ovalbumin and carrageenan solutions were filtered (0.22 μm) and degassed in a vacuum (TA Instruments, Degassing