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Complexation of sodium caseinate with gum tragacanth: Effect of various species and rheology of coacervates

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ABSTRACT

We investigated complex coacervation of sodium caseinate/*Astragalus rahensis* (A.r) as a function of pH with light scattering, spectrophotometry, and viscosity measurements. Interestingly, sodium caseinate/A.r displayed five structural transitions; pH 7.00 to pH ~5.40: no interaction occurred, pH ~5.40 to pH ~4.80: initiation of the formation of primary soluble complexes, pH ~4.80 to ~4.30: formation of interpolymer complexes, pH ~4.30 to ~4.02: optimum coacervation and pH ~4.02 to ~2.50: suppression of coacervation. In addition, rheological properties of sodium caseinate/A.r coacervates were studied at various pH values. A much higher storage modulus (G') than loss modulus (G'') for all sodium caseinate/A.r coacervates suggests the formation of highly interconnected gel-like network structures with mainly elastic behaviour. Moreover, sodium caseinate/A.r coacervates at all pH values exhibited a shear thinning behaviour across the entire shear rate range investigated. Effects of different species of gum tragacanth on the interactions with sodium caseinate have been scarcely studied. Our study showed that systems containing various species (A.r, soluble fraction of A.r and *Astragalus gossypinus* (A.g)) had different critical pH values and particle sizes during complex coacervation, which could be due to different ratio of soluble to insoluble fractions and uronic acid content of various species.

1. Introduction

Proteins and polysaccharides are often jointly present in food and biological systems. The knowledge of the interactions between these biopolymers is important since they are used in various phenomena, from biological systems such as organization of living cells [1] to industrial applications like protein separation and purification [2], microencapsulation [3,4], and processed foods [5]. When solutions of polysaccharides and proteins are mixed together, one may encounter several situations: (1) co-solubility, arise in dilute systems; (2) incompatibility, occurring when there is a repulsive interaction between the biopolymers; and (3) complexation or coacervation, when there is an attractive interaction [5–7]. The general picture for coacervation between protein and anionic polysaccharide is that when pH > p*I*, the "charge patches" on the protein molecules cause the protein to interact with anionic polysaccharides to form soluble protein/polysaccharide complexes, initiated at the first critical pH (pH_C). At the second critical pH ($pH_{\Phi 1}$), soluble protein/polysaccharide complexes start to aggregate into insoluble complexes, which ultimately sediment into the dense coacervate phase due to charge neutralization. The charges neutralization of anionic polysaccharides can also reduce the rigidity of backbone chains as a result of a decrease in repulsive interaction of like-charges groups [6,8–11]. Optimal complex formation is considered to occur at a pH where both biopolymers reach their electrical equivalence, denoted as the third critical pH (pH_{opt}) [12]. When pH decreases to the fourth critical pH ($pH_{\Phi 2}$), protein/polysaccharides complexes dissociate into soluble complexes, or even into uninteracted protein and polysaccharide chains [13]. Because the main attractive force between proteins and polysaccharides is electrostatic interaction, the size and stability of such complexes depends on a number of physicochemical parameters, such as, pH, ionic strength, polysaccharide linear charge density, protein surface charge density, rigidity of polysaccharide chain, and protein/polysaccharide ratio [14–17].

In food industry milk-derived ingredients are increasingly used because of their specific functional properties. Casein represents

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80% of milk proteins and caseinate derivatives are now used both in dairy and non-dairy products [18]. Sodium caseinate is a heterogeneous mixture of caseins, composed of four phosphoproteins: αs_1-, αs_2-, β- and κ-casein and is approximately 10 nm in diameter [19,20]. It dissolves readily in water, when the pH of medium is a little different from the isoelectric point of protein, which is $pI \approx 4.6$ [21]. Previous studies have shown that sodium caseinate can interact with anionic or cationic polysaccharides in aqueous media (e.g. carrageenan, xanthan, locust bean gum (LBG), gum arabic, pectin, chitosan, guar gum, dextran sulfate, sodium alginate and gum tragacanth (A.g)) to form complexes [22–34]. The pH, ionic strength, protein or polysaccharide concentration, charge density as well as processing conditions are the most important factors influencing the formation of these complexes.

Gum tragacanth is a branched, heterogeneous and anionic carbohydrate with a molecular weight of about 840 000 Da. It is defined by the Food Chemical Codex as the dried exudate, which is obtained from the stems and branches of Asiatic species of *Astragalus*. Gum tragacanth consists of two major fractions: water-soluble (tragacanthin) and the water-swellable (bassorin) [35–38]. Tragacanthin is composed of tragacantic acid (ethanol-insoluble) and arabinogalactan as an ethanol-soluble minor fraction [39]. It has been reported that different species of *Astragalus* have different ratios of the two fractions, different chemical compositions (uronic acid contents and different neutral sugars contents), physicochemical and rheological properties. Hence, these differences result in specific functionalities and applications for each species of gum tragacanth [40–43]. The continued use of the gum is the result of its unique properties of acid stability, ease of emulsification, high viscosity, smooth and creamy mouthfeel [44–46]. Our recent work [34] showed that complexation between gum tragacanth (*Astragalus gossypinus* (A.g)) and sodium caseinate was initiated by electrostatic interactions. These interactions resulted in the development of different types of complexes, which their characteristics were dependent on the acidic condition. Hasandokht Firooz et al. [47] parameterized the pH-induced structural transitions of Tragacanthin/β-Lactoglobulin mixture in terms of a set of characteristic pH values by coupling the slow in situ acidification of the Tragacanthin/β-Lactoglobulin mixture and rheometry. There are several reports on the stabilization of fermented dairy products using gum tragacanth [48–50]. Accordingly, it has been suggested that the interactions between milk proteins and gum tragacanth are mainly electrostatic. Manipulating these interactions resulted in improvements in physical stability and texture properties of these products.

Protein–polysaccharide complexes and coacervates are exhibiting a wide range of potential applications as drug carriers [51,52], fat replacers or meat analogues [53], coatings and packaging materials [54]; however, their specific application will depend on their structure and rheological properties. Furthermore, the interaction between protein–polysaccharide will influence the rheological properties of the system. There have been a handful of reports about the rheological properties of individual caseinate solutions [19,55–58] or tragacanth [39,40,59,60] but little is known about rheological properties of tragacanth/caseinate mixtures.

In this work, primarily the interaction between sodium caseinate/*Astragalus rahensis* (A.r) as a function of pH was investigated using spectrophotometry, light scattering and rheometry. Further, the microstructure and rheological features of coacervates formed at various pH values were characterized so that with this information specific future applications of these coacervates can be achieved. Ultimately complex coacervation process of sodium caseinate with different species of gum tragacanth at acidic pH conditions was compared.

2. Materials and methods

2.1. Materials

Two species of Iranian gum tragacanth exuded from *Astragalus rahensis* (A.r) and *Astragalus gossypinus* (A.g) were collected from plants growing in different provinces of Iran (Khorasan and Isfahan respectively). The raw gum was ground and sieved. Powdered gum with a mesh size between 200 and 500 μm was used in this study. Sodium caseinate salt from bovine milk (lot 100M0130V) was purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA) for use in this study.

2.2. Separation of soluble fraction

In order to prepare dispersion, 1 g of whole gum powder was dispersed in distilled water. To assure fully hydration the mixture was stirred at 35 °C for 2 h and left at 4 °C overnight. In order to separate soluble and insoluble parts, the suspension was centrifuged at 10,000 × g for 60 min at 35 °C. The supernatant was freeze-dried at −85 °C for 8 h at 11 Pa and sealed in zip plastic bags and then stored in desiccators.

2.3. Preparation of biopolymer mixed stock dispersions

The sodium caseinate/gum tragacanth mixed dispersions were prepared by adding a sodium caseinate solution to a gum tragacanth dispersion at protein to polysaccharide (Pr:Ps) weight ratios of 2:1 and 3:1 and total biopolymer concentrations of 0.3 wt.% and 0.06 wt.%. 1, 0.1 and 0.01 N HCl (or 0.1 and 0.01 N NaOH, if necessary) solutions were used to acidify the dispersion from pH ∼7.00 to pH ∼2.50. After adjustment of the mixture's pH to the desired value, samples were taken out for spectrophotometry, rheological measurements and particle size analysis.

2.4. Preparation of sodium caseinate/A.r coacervates

The pH of sodium caseinate/A.r dispersions (0.3 wt.%, Pr:Ps 3:1) was set at various values: ∼4.44, ∼4.23, ∼4.04 and ∼3.83. After acidification, the coacervates were collected after removal of supernatant through centrifugation at 4000 rpm for 35 min.

2.5. Spectrophotometry

Absorbance measurements were carried out with a SP-300 PLUS Optima UV-VIS spectrophotometer (Tokyo, Japan) at a wavelength of 400 nm. The samples were put in a 1 cm path length cuvette, and the absorbance (Abs) was measured at 22 ± 2 °C.

For time-dependent spectrophotometric measurements, a volume of 3 ml of a sodium caseinate/gum tragacanth mixed dispersion of the desired pH was put in the cuvette, and the absorbance was followed for 30 min with a measurement step of 30 s.

2.6. Particle size analysis

The particle size distribution of the samples was determined with a laser diffraction particle size analyzer (Cilas 1090, Orleans, France) equipped with a 5 mW He/Ne (635 nm) laser beam. The sample was added to the measuring unit containing distilled water at a pH similar to the pH of the sample. The particle size measurements are reported as $d(0.1)$ μm, $d(0.5)$ μm and $d(0.9)$ μm on a surface basis that is the size of the particle below which 10%, 50%, and 90%, respectively, of the sample particles lie.