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Influence of main whey protein components on the mechanism of complex coacervation with *Acacia* gum

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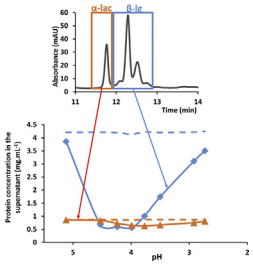
HIGHLIGHTS

- The two main components of whey protein isolate, β-lactoglobulin and α-lactalbumin, undergo complex coacervation with *Acacia* gum.
- β-lactoglobulin has stronger binding to *Acacia* gum than α-lactalbumin.
- A residual concentration of proteins remains in the aqueous phase in all instances.

GRAPHICAL ABSTRACT

Quantification of α-lac and β-lg in whey proteins/*Acacia* gum coacervate by capillary gel electrophoresis.



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ABSTRACT

Complex coacervation between whey proteins isolate (WPI) and *Acacia* gum was investigated in order to disclose the roles and the contributions of each component of WPI to the formation of the complex coacervate. The main aim was to establish the balance of the main components of WPI, β-lactoglobulin and α-lactalbumin, during the phase separation of complex coacervate. The complex coacervation of *Acacia* gum and pure β-lactoglobulin, pure α-lactalbumin, and WPI have been investigated and compared together by means of macroscopic observations and capillary gel electrophoresis analyses performed along pH scans from basic to acidic medium. Coacervate composition was influenced by the protein/polysaccharide (Pr:Ps) ratio and pH. An optimum pH for best coacervation yield was found for each Pr:Ps ratio. Non-negligible concentrations of β-lactoglobulin and α-lactalbumin remain in solution in most instances. β-lactoglobulin undergoes complex coacervation stronger than α-lactalbumin in their competitive coacervation of WPI and *Acacia* gum.

1. Introduction

Proteins and polysaccharides are widely used polymers in the food industry especially in emulsions and encapsulation based products [1–3]. Two different biopolymers mixed together may either form a one-phase or a two-phase system. Biopolymers mixtures often undergo a phase separation. Two types of phase separation can be observed in biopolymers mixtures: the segregative phase separation and the associative phase separation. The segregative phase separation occurs when the protein and the polysaccharide are repelling each other because of their thermodynamic incompatibility, whereas the associative phase separation

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occurs in case of attractive interaction between a protein and a polysaccharide [4–6]. An associative phase separation of electrostatic origin between the two polyelectrolytes of opposite electrical charges takes place in the case of complex coacervation. The complexation of protein and polyelectrolytes is driven by electrostatic interactions; it is controlled by a charge regulation process [7].

The main origin of complex coacervation is an associative phase separation phenomenon induced by electrostatic interactions between at least two macromolecules, typically a protein and a polysaccharide of opposite charges [8]. But other weak interactions, such as hydrogen bonding and hydrophobic interactions, can contribute to the formation of complex coacervates [9]. The associative phase separation induces the formation of neutral soluble complexes which exhibit an attractive interaction [10]. Such complexes undergo precipitation into liquid droplets that possibly settle to form the coacervated phase [11]. Though complex coacervation is a phase separation that can be considered as a precipitation, the complex coacervate particles are extensively swollen by water and better look like microgel particles in suspension in water. After complexation each biopolymer can be found both in the coacervates and to a small extent, in solution in the aqueous phase [12]. Gelatin/*Acacia* gum polymer pair is one of the first and most widely used complex coacervation system [13,14]. Gelatin/*Acacia* gum coacervates exhibit a wide range of useful functionalities for the development of food products such as thickening, gelling, foaming and emulsifying abilities [15]. Coacervates are also used for nano- or microencapsulation of various lipophilic compounds, food ingredients and pharmaceuticals [9,16–18].

Whey proteins isolate/*Acacia gum* (WPI/AG) is another biopolymer system used for complex coacervation, where WPI is used to replace gelatin. It was previously shown that the interaction between WPI and AG is mainly driven by the interaction of β-lactoglobulin (β-lg) with *Acacia* gum [19]. Indeed β-lg is the major component of WPI on the one hand; the second major component is α-lactalbumin (α-lac). In addition, the higher isoelectric point of β-lg (pH 5.2) compared to α-lac (pH 4.1) leads to the formation of β-lg/AG complexes earlier than the α-lac/AG complexes as the pH is lowered from above the isoelectric point [20].

The effect of pH, protein to polysaccharide ratio (Pr:Ps), ionic strength and total biopolymer concentration on complex coacervates formation has been studied in WPI/AG and β-lg/AG systems. For the WPI:AG system, complex coacervation has been observed in a pH range from 3 to 5 for mixtures of 10 wt% biopolymers and a 3:1 Pr:Ps ratio. Only very weak interactions were observed between the two polymers at higher pH (between pH 5 and 7) [21]. The optimum pH of complex coacervation was found at 4.0 for a 2:1 Pr:Ps ratio. At this pH, phase separation occurred the fastest and the final coacervate volume was the largest. Varying the protein to polysaccharide ratio (Pr:Ps) shifted the optimum pH to higher values when Pr:Ps was increased. Such shift of optimum pH was probably due to variations of the concentrations of residual soluble species as the Pr:Ps ratio was varied. It was also shown that increasing the ionic strength led to a less concentrated and poorly structured coacervate phase, induced by the screening of the electrostatic interactions [22]. The electrostatic nature of the interactions between β-lg and *Acacia* gum has also been pointed out according to the pH dependence of the phase separation, together with the influence of the protein to polysaccharide ratio and the total biopolymer concentration [23]. Finally, changes in β-lg/AG complex coacervation were observed in the presence of β-lg aggregates. The simultaneous formation of spherical vesicular coacervates and precipitates were observed when the β-lg solution contained aggregates, whereas only coacervates were observed in aggregate-free β-lg solution. Removal of protein aggregates can be performed by centrifugation (RCF = 10,000 × *g* for 1 h at pH 4.75) [24].

In WPI/AG complex coacervation system three transitions have been observed in pH scan down to acidic medium. The formation of soluble complexes was observed first, thereafter phase separation took place in a second stage, and the last event was the dissociation of the complexes as the isoelectric point of *Acacia* gum is approached [20]. A more detailed picture of complex coacervation has been obtained with the β-lg/AG system. It is described as a nucleation and growth type process where the phase separation is delayed with respect to the formation of the insoluble complexes because a high enough supersaturation is required for nucleation to operate [25]. Evidence of six pH-induced structural transitions has been given during complex coacervation between β-lg and *Acacia* gum at a 2:1 ratio. Characteristic stages are the initiation of the interactions at pH 4.9, the aggregation of complexes at pH 4.6, the nucleation of phase separation at pH 4.4, the massive phase separation at pH 4.2, the observation of large spherical coacervates by optical microscopy at pH 4.0 and morphological changes of coacervates at pH 3.8 [26]. The thermodynamic mechanisms of β-lg/AG binding processes at pH 4.2 include two different binding steps, a first exothermic step and a second endothermic step [27].

The main objective of the present work was to get a definite balance of the coacervation of the main components of WPI with an anionic polysaccharide, *Acacia* gum. Commercial WPI was mainly composed of β-lg and α-lac. The precise composition of the commercial WPI used for this study was firstly determined. Then the formation of complex coacervates was investigated in the WPI/AG system and compared to that of its pure components in the respective β-lg/AG and α-lac/AG systems. The fraction of β-lg and α-lac involved in WPI/AG complex coacervates as a function of pH and biopolymer ratio was determined by capillary electrophoresis.

2. Material and methods

2.1. Materials

Whey protein isolate (Prolacta® 95) was a gift from Lactalis Ingredients (Bourgbarré, France). The powder contained 95% protein with respect to full dry matter. *Acacia* gum (AG), β-lactoglobulin (β-lg), α-lactalbumin (α-lac), and hydrochloric acid were purchased from Sigma–Aldrich. Deionized water of 18 MΩ cm was used.

2.2. Preparation of polymers solutions

Whey proteins isolate (WPI), β-lg, α-lac and *Acacia* gum were dispersed in deionized water and mixed under gentle stirring at room temperature until complete dissolution. The concentrations of WPI solutions were 10 mg mL^{-1} for capillary electrophoresis assays and 100 mg mL^{-1} for SDS-PAGE analyses. Complex coacervation studies were carried out with polymer solutions at 10 mg mL^{-1}.

2.3. SDS-PAGE

The identification of β-lg in WPI was carried out by polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulfate (SDS). 5 samples of 1–3 μL of WPI solution were deposited in five wells and 10 μL of bromophenol blue were added to each samples. Molecular weights were estimated using Fermentas electrophoresis protein standard (PageRuler™ Unstained Protein Ladder). The stacking gel and the separation gel were 10% acrylamide. After electrophoresis, gels were fixed for 30 min, destaining was carried out overnight until the background color has completely disappeared, and images of the destained gels were taken.