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Rheological and structural characterization of agar/whey proteins insoluble complexes

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ABSTRACT

Complex coacervation between whey proteins and carboxylated or highly sulphated polysaccharides has been widely studied. The aim of this work was to characterise a slightly sulphated polysaccharide (agar) and whey protein insoluble complexes in terms of yield, composition and physicochemical properties as well as to study their rheological behaviour for better understanding their structure. Unlike other sulphated polysaccharides, complexation of agar and whey protein at pH 3 in the absence of a buffering agent resulted in a coacervate that was a gel at 20 °C with rheological properties and structure similar to those of simple agar gels, reinforced by proteins electrostatically aggregated to the agar network. The behaviour towards heat treatment was similar to that of agar alone, with a high thermal hysteresis and almost full reversibility. In the presence of citrate buffer, the result was a "flocculated solid", with low water content (75–81%), whose properties were governed by protein behaviour.

1. Introduction

Protein–polysaccharide interactions are the basis for many important biological processes. In foods, proteins and polysaccharides are the most important structure-forming ingredients (Tolstoguzov, 1991) and their use in mixed systems can improve or modify their functional behaviour (Dickinson & Galazka, 1991; Dickinson & Izgi, 1996; Gurov & Nuss, 1986; Turgeon & Beaulieu, 2001).

Mixing a protein with a polysaccharide into an aqueous solution may drive to one of several situations (de Kruif & Tuinier, 2001; Doublier, Garnier, Renard, & Sanchez, 2000; Syrbe, Bauer, & Klostermeyer, 1998; Tolstoguzov, 1991) depending on the polymer–polymer and solvent–polymer attractive or repulsive interactions present and on the polymer molecular masses:

(a) Co-solubility, though this is the least typical situation;
(b) Incompatibility: in this case the system may have one or two phases. In the former, the biopolymers concentrations are lower than the phase-separation threshold. In the latter each phase is enriched in one of the polymers (segregation), when the concentrations are above that threshold;
(c) Complexation: in this case also one or two phase systems can be formed. The soluble complexes are stabilised through electrostatic interactions and hydrogen bonding resulting in one phase system. Two phases are formed when polysaccharides are adsorbed onto the protein or bridge between several protein molecules, therefore concentrating both polymers in one phase leading to the exclusion of the solvent from their vicinity. From the two phases formed one of them is thus enriched in both polymers while the other is depleted in both polymers and solvent enriched. This phenomenon has been called aggregative phase separation or coacervation and is typical of oppositely charged anionic polysaccharides and proteins.

In fact, coacervation between an anionic polysaccharide and a protein is a widely known phenomenon and many examples are described in the literature (e.g. (Burgess, Kwok, & Megremis, 1991; de Kruif, Weinbreck, & de Vries, 2004; Galazka, Smith, Ledward, & Dickinson, 1999; Imeson, Ledward, & Mitchell, 1977; Laneuville, Paquin, & Turgeon, 2000; Mendanha et al., 2009; Onder, Sarier, & Cimen, 2008) due to both their biological relevance and promising industrial applications (Aberkane, Jasniewski, Gaiani, Scher, & Sanchez, 2010; Turgeon, Schmitt, & Sanchez, 2007). These

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applications include encapsulation, protein recovery, multilayer structures (such as packaging films), biomimetic systems and new food gels or emulsions. It is usually a reversible process, driven by a large entropy increase due to the release of counterions, that depends on the properties of the biopolymers involved (charge density, molecular weight, total biopolymer concentration and protein to polysaccharide ratio), on the environmental conditions (pH, ionic strength, type of ions) and on external factors such as temperature, pressure and shearing (Schmitt & Turgeon, 2011). Functional properties of polysaccharide-protein complexes are usually improved, probably by combination of the properties of each component (Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998).

Whey proteins (WP) are widely used in food formulations due to their nutritional and functional properties (de Wit, 1998; Turgeon & Beaulieu, 2001). Besides their classical nutritional benefits, they are well known for their versatile functionality, including emulsifying, gelling or foaming abilities. Whey proteins are the group of milk proteins that remain soluble in "milk serum", or whey, after precipitation of caseins at pH 4.6 and 20 °C. They possess high levels of secondary, tertiary and, in most cases, quaternary structures. Most of them are globular proteins (feature responsible for many of their functional properties) that can be denatured on heating (e.g. completely at 90 °C for 10 min) and contain intramolecular disulphide bonds that stabilise their structure. Their isoelectric point typically varies between 4.5 and 5.5.

Agar is a biopolymer extracted from red seaweeds extensively used in food and pharmaceutical industries as gelling and stabilising agent. It is built up on two main fractions: agarose, the linear neutral fraction responsible for agar's good gelling ability, and agaropectin, the charged polymer fraction that results from the presence of several substituent groups such as sulphates, methyl ethers and pyruvates. Although this polyanionic polysaccharide is a sulphated polysaccharide and has been considered as a strong polyelectrolyte (Boral & Bohidar, 2010), its sulphate content is much lower than in dextran sulphate and carrageenans. This fact can be beneficial when coacervating since, though the charges of polyelectrolytes should be large enough to allow significant electrostatic interactions, too many charges can cause precipitation of the complex instead of coacervation (Boral & Bohidar, 2010). In fact, precipitation has been referred in whey proteins and strong polyelectrolytes complexation, such as sulphated carrageenans or dextran sulphate (de Kruif et al., 2004; Weinbreck, Nieuwenhuijse, Robijn, & de Kruif, 2004).

Numerous studies have focused on whey protein/polysaccharide interactions including complex coacervation applications (e.g. (Aberkane, Jasniewski, Gaiani, Hussain, Scher, & Sanchez, 2012; Weinbreck, Tromp, & de Kruif, 2004)). One particular application is the separation of β-lactoglobulin and α-lactalbumin (e.g. Hidalgo & Hansen, 1971), as they have slightly different isoelectric pH values (5.2 and 4.1, respectively). At pH 4.5, and under adequate conditions, β-lactoglobulin forms a coacervate and α-lactalbumin remains in solution (Capitani, Pacheco, Gumerato, Vitali, & Schmidt, 2005). Other applications include their use as functional agents (stabilisers, fat replacers, texturising agents) or microencapsulation. Coacervation between a sulphated polysaccharide and a globular protein has been described in the literature (e.g. (Jones, Handschin, Adamcik, Harnau, Bolisetty, & Mezzenga, 2011; Klassen, Elmer, & Nickerson, 2011; Weinbreck, Nieuwenhuijse, et al., 2004; Weinbreck, Tromp, et al., 2004)). However, applications involving agar complexation are very rare and, to our knowledge, mostly gelatine-agar systems have been studied (Boral & Bohidar, 2010; Singh, Aswal, & Bohidar, 2007, 2011), being gelatine a structural non-globular protein, unlike e.g. whey proteins. A previous study on the interactions between bovine whey protein isolate (WPI) and agars (commercial or obtained by microwave-assisted extraction) with different physicochemical properties was performed by the authors' group, using spectroscopic and calorimetric techniques (Souza, Sousa, Gómez, & Gonçalves, 2012). Optical dispersion (O.D.) and isothermal titration calorimetry (ITC) results revealed that molecular mass and sulphate content of different agars had a determinant effect on coacervate formation. No studies on WPI/agar insoluble complexes' characterization were made. Therefore, the present paper aims at characterizing a low sulphate content polysaccharide (agar) and WPI insoluble complexes in terms of yield, composition and physicochemical properties as well as studying their rheological behaviour in order to better understand their structure.

2. Materials and methods

2.1. Materials

Whey protein isolate (WPI) was obtained as a commercial sample (Lacprodan DI-9224) from Arla Foods Ingredients, Ambh (Denmark), and used as protein source without further purification. As specified by the manufacturer, the isolate contains a minimum of 92% total protein content, and the major protein constituents are: 74% β-lactoglobulin (β-LG, 18.36 kDa), 18% α-lactalbumin (α-LA, 14.5 kDa), 6% bovine serum albumin (BSA, 69 kDa). The isolate further contains lactose and fat (each at a maximum content of 0.2%), and minerals such as sodium (0.5%), potassium (1.3%), and calcium (0.1%). Commercial agar (ref. A-7002 with an ash content of 2–4.5%) was obtained from Sigma-Aldrich Co. (St. Louis, MO). The average molecular mass (M_v) of the polysaccharide was obtained from viscosity measurements in a previous work (Souza et al., 2012) and was estimated to be 137.5 kDa.

All other chemicals (citric acidic, sodium citrate, sodium chloride, etc.) were of analytical grade and used without further purification.

2.2. Preparation of solutions

Commercial agar and WPI stock solutions were separately prepared by dissolving the amounts of their corresponding solids in 10, 20 or 50 mmol dm^{-3} citrate buffer or in water. When necessary, pH (measured using a Crison pHmeter, model GLP 21, Spain) was adjusted to 3 by addition of citric acid, sodium citrate or, in the case of water solutions, with concentrated hydrochloric acid.

Agar dispersions were heated at 95 °C for 30 min with gentle stirring to ensure complete dissolution. They were subsequently incubated at 40 °C to avoid gelation, till further use. WPI dispersions were gently stirred at room temperature till complete dissolution and left to hydrate for 6 hours before use.

2.3. Turbidity measurements

Turbidity (optical dispersion, OD) of mixed WPI/agar dispersions was measured using a UV–visible spectrometer (Jasco, V-630 Bio), at a wavelength of 400 nm using quartz cells with a light path of 10 mm. Turbidity values were only recorded when the signal became stable and were corrected using buffer as blank. Each reported value is the average of three consecutive readings. In order to study the effect of WPI concentration on the extent of coacervate formation with agar, several solutions were prepared in 2 mL disposable centrifuge tubes containing a constant concentration of agar (0.1%, w/w) and increasing concentrations of protein delivered from a common stock solution (0.4%, w/w). The mixed dispersions were prepared at pH 3 and 35 °C and were allowed to stand at this temperature for at least 30 min to ensure that the reaction was completed before recording their turbidity. Three replicas were made for each buffer.