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Electrostatic adsorption and stability of whey protein–pectin complexes on emulsion interfaces

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ABSTRACT

The objectives of this study were, firstly, to adsorb biopolymer complexes to an emulsion interface through electrostatic interactions and, secondly, to test the stability of the emulsions covered with biopolymer complexes. Whey protein isolate (WPI)–apple pectin complexes made by thermal treatment (85 °C, 20 min) were successfully adsorbed to the interface of an oil-in-water emulsion stabilized with whey proteins. The stability of the emulsion covered with the WPI-pectin complexes was tested by measuring salt (0–500 mM NaCl), heat (40–90 °C, 30 min) and freeze-thaw (−20 °C, 22 h) stability at pH 3.5–4.5. The results revealed that the adsorption of WPI-pectin complexes to the emulsion interface led to the formation of stable emulsions. The most remarkable result was at pH 4.5, where the base emulsion (without biopolymer complexes) was aggregated, but became stable after the deposition of the WPI-pectin complexes. Emulsions covered with WPI-pectin complexes were stable to salt additions up to 200 mM, but aggregated at the 500 mM level of NaCl. They were also resistant to heat treatments, and no aggregation was observed. However, the adsorption of WPI-pectin complexes to the emulsions did not improve the freeze-thaw stability, on the contrary, they showed major aggregation. These results demonstrated that biopolymer complexes can be used to assemble hierarchial emulsion structures and, improve the emulsion stability to environmental stresses.

1. Introduction

Biopolymer complexes and coacervates have emerged as a potential tool to produce specific microstructures and novel functionalities in the food, pharmaceutical and cosmetic industries. A prerequisite for the successful use of protein-polysaccharide complexes is that they need to exhibit the rheological and textural properties desired, as well as interfacial properties, when incorporated into the chosen application. Ideally, these biopolymer complexes should also enhance (food) quality and nutritional attributes, as well as the bioavailability or absorption of active substances (Turgeon, Beaulieu, Schmitt, & Sanchez, 2003).

The interactions between biopolymers (proteins and polysaccharides) in solutions have been under extensive investigation and review (Burgess & Carless, 1984; Doublier, Garnier, Renard, & Sanchez, 2000; de Kruif, Weinbreck, & de Vries, 2004; Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998; Turgeon et al., 2003). In general, mixtures of biopolymers lead to either phase separation through thermodynamic incompatibility (biopolymers segregate into separate phases) or complex coacervation (biopolymers associate, excluding solvent from their vicinity). Segregative phase separation occurs at high concentrations and high ionic strengths when both biopolymers carry similar charges, thereby resulting in electrostatic repulsion between the molecules. On the other hand, associative phase separation or complex coacervation usually occurs at relatively dilute concentrations, low ionic strengths and when both biopolymers carry opposite charges. Thus, the electrostatic attraction between the associating biopolymers occurs at a pH between the isoelectric point (pI) of the protein and the pK_a of the polysaccharide (Burgess & Carless, 1984; Doublier et al., 2000; de Kruif et al., 2004; Schmitt et al., 1998; Turgeon et al., 2003).

Associative phase separation of natural and synthetic polymers has potential applications especially in encapsulation and delivery systems using micelles (Cohen Stuart, Besseling, & Fokkink, 1998; Lindhoud, de Vries, Schweins, Cohen Stuart, & Norde, 2009; Voets, de Keizer, & Cohen Stuart, 2009), microemulsions (Hofs et al., 2008) or vesicles (Renard et al., 2002). Previous research has shown that biopolymer complex/coacervate cores can be used, for example, as drug carriers (Lankalapalli & Kolapalli, 2009; Luzzi, 1970;

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Thomasin, Nam-Trân, Merkle, & Gander, 1998) and packaging material for enzymes (Nahalka, Dib, & Nidetzky, 2008; Pommersheim, Schrezenmeir, & Vogt, 1994) as well as to produce films (Mendelsohn et al., 2000) and reversible gels (Lemmers, Sprakel, Voets, van der Gucht, & Cohen Stuart, 2010). Other interesting functions for polymer complexes include microgels that can entrap and release charged molecules upon heating (Ohsugi, Furukawa, Kakugo, Osada, & Gong, 2006). They can deliver oils and flavors in food and drinks (Gouin, 2004) and act as fat replacers (Laneuville, Paquin, & Turgeon, 2005).

Protein-stabilized emulsions are usually prone to instabilities by pH, ionic strength and temperature (Kim, Decker, & McClements, 2002; McClements, 2005a). The interfacial layers formed by proteins are usually relatively thin and electrically charged, and thus, the major mechanism preventing droplet flocculation in protein-stabilized emulsions is electrostatic repulsion, rather than steric repulsion. The developments in bilayer or multilayer assembly have strongly influenced the stability of emulsions (Cooper, Dubin, Kayitmazer, & Turksen, 2005). The driving force for creating these hierarchical structures is that electrically charged biopolymers are capable of adsorbing to the surfaces of oppositely charged emulsion droplets through electrostatic interactions (Dickinson, 2003). Studies have demonstrated that droplets coated with multilayered interfacial membranes often have improved stability to thermal treatment, freezing, drying, and mechanical agitation compared to the single-layered emulsions because of the increase in interfacial thickness and rheology (Aoki, Decker, & McClements, 2005; Gu, Decker, & McClements, 2007; Guzey & McClements, 2006a; 2006b). There are alternative ways of assembling biopolymer layers composed of protein-polysaccharide complexes at the oil-water interface: (1) Polysaccharides can be added to the interface by layer-by-layer assembly, where the polysaccharide solution is added to a previously formed protein monolayer (e.g., a protein-stabilized emulsion) (Dickinson, 2009; Ganzevles, Zinoviadou, van Vliet, Cohen Stuart, & de Jongh, 2006; Guzey & McClements, 2006a), (2) Complex previously formed in solution (containing both the protein and polysaccharide) can be adsorbed to a bare interface (e.g., oil) (Jourdain, Leser, Schmitt, Michel, & Dickinson, 2008), or (3) A mixed biopolymer solution can be adsorbed to a previously formed protein monolayer (Moschakis, Murray, & Biliaderis, 2010). Chitosan and gum arabic aggregates, for example, have been adsorbed to protein-stabilized oil-in-water emulsions (Moschakis et al., 2010), and mixed dextran sulfate and sodium caseinate solution has been homogenized with oil (Jourdain et al., 2008)

Thus, the formation of (bio)polymer particles that respond in desired ways to changes in temperature, pH, ionic strength, or shearing is determined by the structural characteristics of the (bio) polymers, as well as by the method preparation. Previously, complexes and coacervates stable to pH, heat treatment and salt additions have been developed from various biopolymers, for example, from β-lactoglobulin–pectin (Jones, Decker, & McClements, 2009; Jones, Decker, & McClements, 2010a; Wang, Lee, Wang, & Huang, 2007), gelatin–pectin (Gilsenan, Richardson, & Morris, 2003), gelatin–acacia (Burgess & Carless, 1984), pea protein–gum arabic (Liu, Low, & Nickerson, 2009), and whey protein–xanthan gum (Laneuville et al., 2005).

Consequently, these biopolymer conjugates and complexes may provide improved stabilization of emulsions (Dickinson, 2009). Therefore, the purpose of this study was to create novel hierarchical structures by using biopolymer complexes. First, we hypothesized that biopolymer complexes can be adsorbed to the emulsion interface through electrostatic interactions. For this study, we formed biopolymer complexes made from WPI and apple pectin with 50% degree of esterification (DE), because of their small size (~250 nm) and good pH stability (Salminen & Weiss, 2013). The second hypothesis was that we expected the adsorbed WPI-pectin complex layer to improve the physical stability of the emulsion against changes induced by salt, heat and the freeze-thaw cycle.

2. Materials and methods

2.1. Materials

Food grade whey protein isolate (WPI) (DSE 9273, dry matter 99.0%, protein 93.9%, lactose < 0.5%, fat 0.2%, moisture 5.2%, ash 1.8%) was donated by Fonterra GmbH (Hamburg, Germany). WPI was manufactured by ion exchange and ultra-filtration according to the manufacturer. WPI was mainly composed of a mixture of β-lactoglobulin A (46.1%), β-lactoglobulin B (43.9%), and α-lactalbumin (9.7%). The pI of WPI was 4.7. The mineral content and composition was Na 0.48%, S 0.24%, Ca 0.07%, K 0.05%, P 0.03%, Fe 0.0003%, Al 0.00001%, and Se 0.0000006%. Apple pectin (Pectin Classic AU606, DE 50%) was donated from Herbstreith & Fox KG (Neuenbürg/Württ, Germany). Pectin contained 83% galacturonic acid and had a molecular weight of ~45 kDa according to the manufacturer. The ratio of methylesterified galacturonic acid groups to total galacturonic acid groups is termed as the degree of esterification (DE). It contained the following minerals: Ca 0.43%, Na 0.24%, S 0.10%, P 0.09%, K 0.06%, Mg 0.03%, Fe 0.02%, Al 0.003%, Zn 0.0007%, and Mn 0.0005%. Medium chain triacylglycerides (chain length C_8 and C_{10}) Miglyol 812 oil was donated by Sasol GmbH (Witten, Germany). Sodium acetate anhydrous was obtained from Merck (Darmstadt, Germany) and glacial acetic acid from Carl Roth GmbH + Co. KG (Karlsruhe, Germany). Sodium azide was obtained from Sigma–Aldrich (Steinheim, Germany). Sodium chloride was obtained from VWR (Haasrode, Belgium). Distilled, deionized water was used throughout the study.

2.2. Biopolymer complexation

2.2.1. Biopolymer solution preparation

Powdered WPI and apple pectin were dissolved in 10 mM sodium acetate (pH 7.0) containing sodium azide (0.02% w/v) as an antimicrobial agent, and stirred at ambient temperature for at least 4–8 h. After the protein and pectin were found to reduce the pH after solubilization, the solutions were adjusted back to pH 7.0 using 1.0 and 0.1 N sodium hydroxide solutions before being mixed together. After mixing, the final protein concentration in the solution was 0.5% (w/w), while the polysaccharide concentration was 0.25% (w/w). Thus, the biopolymer complex solution had a total biopolymer concentration of 0.75%.

2.2.2. Biopolymer complex formation

The WPI (0.5% w/w)–pectin (0.25% w/w) solution was adjusted to pH 4.75 and stirred for 30 min. Then the mixed solution was heat-treated (85 °C, 20 min) and cooled at room temperature for 2 h.

2.3. Emulsion preparation

An emulsifier solution containing 1.0% (w/w) protein was prepared by dispersing WPI into water containing 0.02% (w/v) sodium azide as an antimicrobial agent, and stirring for at least 2 h to ensure complete hydration. A primary emulsion was prepared by homogenizing 10% (w/w) Miglyol oil with 90% (w/w) emulsifier solution (1.0% WPI in water) with a standard unit homogenizer (Labworld, Staufen, Germany) at high speed (24,000 min^{-1}) for 2 min followed by four passes through a two-stage high-pressure homogenizer (APV-2000, APV, SPX Brand, Albertslund, Denmark):

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