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Influence of sulphate, chloride, and thiocyanate salts on formation of β-lactoglobulin–pectin microgels

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ABSTRACT

Effects of sulphate, chloride, and thiocyanate salts on the heat-induced formation of protein-based microgels from β-lactoglobulin–pectin complexes were determined as a function of pH and protein-to-polysaccharide ratio. Aggregation temperatures were initially decreased at low ionic strength due to shielding of electrostatic interactions between β-lactoglobulin and pectin but increased with further increases in ionic strength. Turbidity of heated mixtures and associated sizes of formed microgels were increased with up to 75 mmol kg^{-1} ionic strength. Aggregation and microgel formation were relatively increased in the presence of thiocyanate salts compared to chloride salts and relatively decreased in the presence of sulphate salts, indicating that the inverse Hofmeister series was relevant in this system. Topographical analysis of dried microgels by atomic force microscopy verified that microgels were smallest in the presence of sulphate salts and showed that added ions, particularly thiocyanate, increased the deformability of microgels during drying.

1. Introduction

Microgels or nanogels are particulate assemblies composed of polymers in a network that retains a significant degree of solvent interactivity, allowing them to expand or contract (i.e. swell) in response to solvent conditions (Oh, Lee, & Park, 2009). Ability of microgels to swell translates to their function as controlled delivery vehicles, their capacity to modify rheological properties (Adams, Frith, & Stokes, 2004), or their behaviour at interfaces (Destribats et al., 2011). Protein-based microgels may be formed from whey proteins by thermal treatment at pH values between 5.8 and 6.2 with radii of 70–200 nm (Schmitt et al., 2010). These whey protein microgels are stable at high or low pH due to cross-linking from covalent disulphide exchanges, as well as hydrophobic interactions; however, the microgels aggregate near their isoelectric point (pH ~5) (Phan-Xuan et al., 2011; Schmitt et al., 2010). Alternatively, microgels of ~200–400 nm are assembled from thermally treated electrostatic complexes between β-lactoglobulin (Blg), the predominant protein within whey, and pectin at pH 4.5–5.0 (Jones & McClements, 2011). Pectin is an anionic polysaccharide isolated from plant cell walls (Yapo, 2011), and this anionic charge provides stability to the microgels over a wide pH range, as it resides on the external surface of the formed microgels (Jones, Decker, & McClements, 2010b).

Effective reduction in electrostatic interactions between proteins and charged polysaccharides is related to the ionic strength parameter (I), which is a function of both total ion concentration and valency (Israelachvili, 2011). Increased ionic strength has been shown to reduce electrostatic interactions between Blg and pectin (Sperber, Schols, Cohen Stuart, Norde, & Voragen, 2009). Since the formation of heated complex microgels is influenced by the interactions between Blg and pectin, increased ionic strength in Blg–pectin mixtures increases the size of formed microgels (Jones & McClements, 2008, 2010). Ion concentration has also been found to decrease the swelling (i.e. dehydration) of already-formed microgels by reducing repulsive internal interactions (Daly & Saunders, 2000; Sağlam, Venema, de Vries, Sagis, & van der Linden, 2011) and to increase the size and stability of Blg microgels in low pH conditions (Jones & McClements, 2008).

Apart from contributing to the ionic strength, different species of ions modify intermolecular interactions and colloidal surface hydration by what is termed "specific ion" or "Hofmeister" effects (Parsons, Bostrom, Lo Nostro, & Ninham, 2011). Different ion species influence the stability and solubility of colloidal systems in

Abbreviations: Blg, β-lactoglobulin protein; KCl, potassium chloride; K_2SO_4, potassium sulphate; NaCl, sodium chloride; NH_4SCN, ammonium thiocyanate; $(NH_4)_2SO_4$, ammonium sulphate; AFM, atomic force microscopy; T_{agg}, aggregation temperature.

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water by changing the hydration and surface tension of the colloidal surface (Collins, Neilson, & Enderby, 2007). The typical ordering of anions in the direct specific ion series (in terms of increasing protein solubility) is given by: carbonate > sulphate > chloride > nitrate > iodide > thiocyanate, with chloride considered a "neutral" anion in terms of specific behaviour (Kunz, 2010; Zhang & Cremer, 2006). Specific cation effects are typically much weaker but follow the order (in terms of increasing protein solubility): ammonium > caesium > potassium > sodium > lithium (Kunz, 2010; Parsons et al., 2011). For positively charged biopolymer, such as proteins, the above series is often reversed (i.e. inverse Hofmeister series) (Zhang & Cremer, 2009). Direct versus inverse series behaviour of ions appears to be related to the binding or exclusion of these ions from charged residues on the colloidal surfaces (Lund & Jungwirth, 2008; Paterová et al., 2013), which may be influenced by the presence of hydrophobic regions, as well (López-León, Santander-Ortega, Ortega-Vinuesa, & Bastos-González, 2008).

Hydration of microgels contributes to their observed size (swelling) and is affected by both ionic strength and specific ion effects (Zhang, Furyk, Bergbreiter, & Cremer, 2005). For instance, thiocyanate and bromide anions were shown to increase the hydration of neutral/anionic hydrogels (Daly & Saunders, 2000; Dodoo, Steitz, Laschewsky, & von Klitzing, 2011; Zha, Hu, Wang, Fu, & Luo, 2002) and decrease hydration of cationic hydrogels (Swann, Bras, Topham, Howse, & Ryan, 2010). Similar specific ion effects were observed at ionic strength values as low as 10 mM for the hydration of poly-*N*-isopropylacrylamide microgels (López-León, Elaïssari, Ortega-Vinuesa, & Bastos-González, 2007). Protein gels are also strongly influenced by specific ion effects (Lawal, 2006; Melnyk, Dreisoerner, Bonomi, Marcone, & Seetharaman, 2011). However, apart from a recent investigation on the influence of calcium and sodium cations (Phan-Xuan et al., 2014), no studies have determined the influence of different salts on the formation and attributes of protein-based microgels.

Microgels formed from Blg involve aggregation at temperatures above its denaturation temperature, which is typically observed at ~75–80 °C (Baeza & Pilosof, 2002). Electrostatic interaction of Blg with polysaccharides do not significantly influence denaturation temperatures at pH 4.75 (Jones, Decker, & McClements, 2010a). Salts, on the other hand, will either increase (e.g. sulphate salts) or decrease (e.g. thiocyanate salts) denaturation temperatures of proteins by decreasing or increasing surface hydration, respectively (Tadeo, Pons, & Millet, 2006). Aggregation of Blg near the isoelectric point occurs in both the native and denatured state, and aggregation between native Blg molecules is driven by electrostatic interactions between local surface regions of opposing charge, which are screened by increasing the ionic strength (Yan et al., 2013). Blg aggregation below 75 °C is also reduced by electrostatically-interacting polysaccharides (Jones et al., 2010a). Thus, factors of ionic strength, salt type, and interactive polysaccharides have potential influences on the aggregation of Blg to form microgels.

The objective of this investigation was to determine the role of specific ion effects on the formation of microgels from heated electrostatic complexes of Blg and pectin, which is an established microgel system (Jones et al., 2010b). In order to efficiently validate specific ion influences in this study, sulphate, thiocyanate, and ammonium salts were chosen to represent the influence of typical kosmotropic, chaotropic, and neutral anions, respectively, as discussed in the literature (Collins et al., 2007). Similarly, ammonium, potassium, and sodium cations of these salts were also used in this study to represent the influences of typical kosmotropic, chaotropic, and neutral cations, respectively. It was hypothesised that for all types of salt the size of microgels would increase with increasing ionic strength, yet the size would be relatively increased or decreased in the presence of these different added salts that could be related to their chaotropic or kosmotropic nature.

2. Materials and methods

2.1. Solution preparation

β-Lactoglobulin (Blg, lot # JE 001-0-415), with a reported composition of 93.6% protein (91.5% Blg), 0.2% fat, 1.8% ash, and 4.4% moisture, was donated by Davisco Food International (Le Sueur, MN). Protein was further purified based on a published methodology (Jung, Savin, Pouzot, Schmitt, & Mezzenga, 2008). Briefly, a 10% (w/w) Blg solution was adjusted to pH 4.60 and centrifuged (15,344×*g*) for 30 min at 20 °C. Supernatant was removed, adjusted to pH 7, and dialysed (MWCO = 6.8 kDa) against ultrapure water for a total of 120 h. Dialysed protein solution was then lyophilised and stored dry until use.

High purity, high methoxy pectin (stated degree of esterification = 52%; sample #3675) was kindly donated by CPKelco (Atlanta, GA) and used without further purification. NaCl, KCl, KSCN, NH_4SCN, K_2SO_4, $(NH_4)_2SO_4$, NaOH, and HCl were obtained from Sigma Chemical Co. (St. Louis, MO). All solutions were prepared with ultrapure water ($\sigma \geqslant 18$ m Ω-cm) obtained from a water filtration system (Barnstead E-pure, Thermo Scientific, Waltham, MA).

Solutions of purified 1.0% Blg, 0.5% pectin, and 0.5 mol kg^{-1} salts (NaCl, KCl, KSCN, NH_4SCN, K_2SO_4, $(NH_4)_2SO_4$) were dissolved in 10 mmol kg^{-1} sodium acetate buffer for a minimum of 3 h. Solutions were mixed at pH 7.0 to obtain a Blg concentration of 0.1% (w/w), a pectin concentration of 0.05% (w/w), and salt concentrations between 0 and 100 mmol kg^{-1}. After mixing, solution pH was reduced by 0.1 N HCl solution. 5-ml aliquots at select pH values were heated in glass tubes at 80 °C for 15 min using a circulating water bath and immediately submerged in an ice-water bath for 20 min. Heated samples were either analysed on the same day or stored overnight at 4–6 °C.

2.2. Sample characterisation

ζ-Potential of proteins and polysaccharides in water was determined by laser Doppler micro-electrophoresis using a Zetasizer Nano ZS light scattering instrument (Malvern, Worcestershire, United Kingdom) at a scattering angle of 173°.

Turbidity of biopolymer solutions was determined from the transmittance of visible light (λ = 450 nm) using an ultraviolet/visible (UV–Vis) spectrophotometer at 25 °C (Lambda 25, PerkinElmer, Waltham, MA). Samples were measured within disposable polystyrene semi-micro cuvettes with a path length of 1.0 cm and an internal volume of 1.4 ml. Buffer was used as a reference blank. Turbidity is presented as 100 – %*T*, where $T = I/I_0$, I and I_0 are the light intensities transmitted through the sample and reference blank, respectively.

Turbidity as a function of temperature was performed with a six-chamber peltier heating block accessory counter-balanced with a water circulation system (Perkin Elmer, Waltham, MA). Background turbidity was subtracted using a separate cuvette within the heating block that was filled with buffer. Aggregation temperature was calculated from the maximum slope of turbidity vs. temperature (i.e. inflection point) for each sample, as performed in the literature (Jones et al., 2010a).

Particle size was determined by dynamic light scattering using a Zetasizer Nano ZS (Malvern, Worcestershire, United Kingdom) at 25 °C with a scattering angle of 173°. Sizes were reported as a *Z*-average diameter.

Atomic force microscopy (AFM) images of dried microgel samples were obtained on a MFP-3D AFM (Asylum Research, Santa Barbara, CA) in intermittent contact mode with a rectangular silicon nitride cantilever (k = 2 N/m, $f_o \cong 175$ kHz). Samples for AFM