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Effects of the conjugation of whey proteins with gellan polysaccharides on surfactant-induced competitive displacement from the air-water interface

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ABSTRACT

Whey proteins can be used to stabilize foams and emulsions against coalescence because of their ability to form viscoelastic films at the interface that resist film rupture on collision between colloidal particles. However, whey proteins are competitively displaced from the interface if small-molecule surfactants are added, leading to destabilization of the entire system. This is because surfactants are more effective in molecular packing at the interface, and they lower interfacial tension to a greater degree than whey proteins do, but their interfacial films are poor in viscoelasticity. We hypothesized that whey proteins would become more resistant to surfactant-induced competitive displacement if they were conjugated with network-forming polysaccharides. The protein moiety of the conjugate would be expected to enable its adsorption to the interface, and the polysaccharide moiety would be expected to form self-assembled networks, strengthening the interfacial film as a whole. In this study, whey proteins were conjugated with gellan polysaccharides using the Maillard reaction. Atomic force microscopy images of interfacial films formed by the whey protein–gellan conjugate at the air-water interface and transferred onto mica sheets using the Langmuir-Blodgett method revealed that gellan did form self-assembled networks at the interface and that interfacial films also contained a large number of unconjugated whey protein molecules. Following the addition of a small-molecule surfactant (Tween 20) to the sub-phase, surface pressure increased, indicating spontaneous adsorption of surfactants to the interface. Atomic force microscopy images showed decreases in interfacial area coverage by whey proteins as surface pressure increased. At a given surface pressure, the interfacial area coverage by whey protein–gellan conjugates was greater than coverage by unconjugated whey proteins, confirming that whey proteins became more resistant to surfactant-induced displacement after conjugation with gellan. Furthermore, gellan molecules added to the sub-phase after the formation of a monolayer of whey proteins at the air-water interface did not adsorb to the interfacial protein film. These results provide a molecular basis for designing interfacial structures to enhance the stability of colloidal systems.
Key words: whey protein, gellan, conjugate, foam, emulsion

INTRODUCTION

Whey proteins can act as stabilizers for colloidal systems such as foams and emulsions, because their amphiphilic nature allows them to spontaneously adsorb to the air-water or oil-water interface and form viscoelastic interfacial films (Dickinson, 2001; Damodaran, 2005). Such films resist rupture, leading to coalescence on collision between colloidal particles. In contrast, the viscoelastic properties of interfacial films formed by small-molecular-weight surfactants are poor (Roth et al., 2000). Therefore, protein-stabilized colloidal systems are generally more stable. Furthermore, the elastic modulus of the interfacial film from the major whey protein β-LG has been reported to be greater than that of β-CN (Mackie et al., 2001). The higher viscoelastic character of the interfacial film of the β-LG can be attributed to higher packing density and stronger protein-protein interactions at the interface, compared with the loose packing and weaker protein-protein interactions of the much more flexible casein molecules (Dickinson, 2001).

The critical bulk concentration of whey proteins at which the interfacial tension reaches a plateau is much lower than that of small-molecule surfactants because of the much larger adsorption energy of whey protein molecules (De Feijter et al., 1987). However, at relatively high bulk concentrations, interfacial tension is lowered to a greater degree by surfactants than by whey proteins, because surfactants are more effective in molecular packing at the interface (Dickinson, 2001; Damodaran 2005). Consequently, as surfactant concentrations increase, whey proteins are competitively displaced from the interface. An orogenic displacement

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mechanism has been proposed based on atomic force microscopy (**AFM**) studies to explain such surfactant-induced competitive displacement (Mackie et al., 1999; Woodward et al., 2004, 2010a). The initial interfacial whey protein film contains void spaces due to packing limitations, allowing small-molecule surfactants to adsorb into these spaces, and then these nucleated sites expand, driven by the difference in surface pressure between surfactant and protein domains. As a result, the continuous protein film is laterally compressed, leading to an increase in film thickness. At sufficiently high surface pressures, the continuous protein film fails, and protein molecules finally desorb into the bulk phase.

An implication of the orogenic displacement mechanism is that the resistance of interfacial protein films to surfactant-induced competitive displacement can be improved by increasing its mechanical strength to make it more resistant to lateral compression (Morris et al., 2011). Dickinson and Hong (1994) treated β-LG with heat before preparing an oil-in-water emulsion, and they found that the heat-treated whey protein was more resistant to displacement from Tween 20, a water-soluble nonionic surfactant. Woodward et al. (2010b) prepared oil-in-water emulsions stabilized with sodium caseinate and treated the emulsion with transglutaminase to catalyze the formation of cross-links between protein molecules. They found that the rate of lipolysis in simulated duodenal conditions decreased significantly as a result of the transglutaminase-induced cross-linking of protein molecules. Their results suggested that a cross-linked interfacial film of casein proteins would be more resistant to displacement by bile salts. Li et al. (2011) also succeeded in reducing the rate of lipolysis by adopting a layer-by-layer electrostatic deposition approach: the formation of a protein layer on the oil droplet surface, followed by the deposition of a secondary layer of an anionic polysaccharide, alginate, on top of the protein layer.

An alternative approach to enhancing the resistance of interfacial protein films to surfactant-induced competitive displacement is to conjugate proteins with polysaccharides that can self-assemble to form networks. Polysaccharides can be conjugated with whey proteins using the initial stage of Maillard reactions, forming covalent linkages between nucleophilic amine groups of proteins and reducing ends of polysaccharides (Nagasawa et al., 1996; Akhtar and Dickinson, 2003; Zhu et al., 2008). The protein moiety of the conjugate is then expected to enable the conjugate to adsorb to the interface because of its surface activity, and the polysaccharide moiety forms self-assembled networks at the interface. The additional polysaccharide network is seen to increase the mechanical strength of the interfacial film as a whole, and therefore its resistance against the expansion of interfacial surfactant domains. In contrast, conjugation with polysaccharides that do not form self-assembled networks would have little effect on surfactant-induced displacement behavior, because such polysaccharides are unlikely to strengthen interfacial films. The objective of this study was to investigate the effect of conjugation between whey proteins and network-forming polysaccharides on the surfactant-induced competitive displacement of whey proteins from the air-water interface. Gellan, a food-grade gelling polysaccharide, was conjugated with whey protein because its molecular structure and properties have been well characterized and reported in the literature (Chandrasekaran et al., 1992; Ikeda et al., 2004a, 2013).

MATERIALS AND METHODS

Materials

Whey protein isolate (BiPRO, lot JE 226-2-420) was supplied by Davisco Foods International Inc. (Le Sueur, MN). The protein content of the dry powder was >90% (wt/wt) according to the manufacturer's specifications. Polyoxyethylene (20) sorbitan monolaurate (Tween 20) was obtained from Sigma-Aldrich (St. Louis, MO). Deacylated gellan (Kelcogel, lot 1J1780A) was provided by CP Kelco (Atlanta, GA). Other chemicals used in this study were of analytical grade.

Preparation of Whey Protein–Gellan Conjugates by Heat Treatment

The protein and polysaccharide were conjugated via the Maillard reaction, induced by heating of the mixtures under controlled humidity (Akhtar and Dickinson, 2003, 2007). Whey protein isolate and gellan were dissolved separately in deionized water, and then mixed at a weight ratio of 1 part whey protein isolate to 2 parts gellan. The mixed solution was adjusted to pH 7.0 and then lyophilized. The lyophilized sample was placed in a preheated desiccator containing a saturated solution of KCl to achieve a relative humidity of 79%, and then it was incubated at 80°C for 2 h. The heat-treated product was adjusted to pH 5.0 and then centrifuged at 7,000 × *g* for 50 min to precipitate heat-denatured whey proteins (Bund et al., 2012). The supernatant was adjusted to pH 7.0. Protein components in the supernatant (including protein–polysaccharide conjugates) were precipitated by adding ammonium sulfate to a final concentration of 5 *M*. The precipitate was dissolved in distilled water and then dialyzed for over 24 h using a Spectra/Pro 6 dialysis membrane with a molecular-weight cutoff of 50 kDa (Spectrum, Rancho Dominguez, CA) and an approximately 40-fold volume