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Dynamic and viscoelastic interfacial behavior of β-lactoglobulin microgels of varying sizes at fluid interfaces

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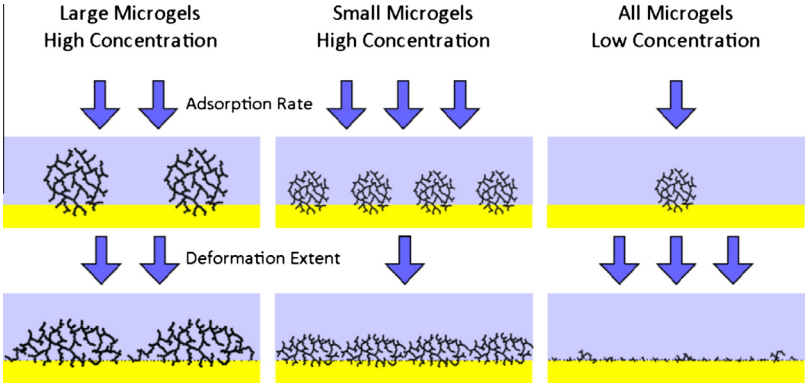
HIGHLIGHTS

- β-Lactoglobulin microgel particles adsorbed and rearranged at heptane–water interfaces.
- Microgel-stabilized interfaces are exhibit strongly elastic characteristics.
- Smaller microgels adsorbed and increased interfacial elasticity faster.
- Microgels converted to smaller aggregates at low interfacial concentrations.

GRAPHICAL ABSTRACT



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ABSTRACT

Hypothesis: Microgel particles formed from the whey protein β-lactoglobulin are able to stabilize emulsion and foam interfaces, yet their interfacial properties have not been fully characterized. Smaller microgels are expected to adsorb to and deform at the interface more rapidly, facilitating the development of highly elastic interfaces.

Methods: Microgels were produced by thermal treatment under controlled pH conditions. Dynamic surface pressure and dilatational interfacial rheometry measurements were performed on heptane–water droplets to examine microgel interfacial adsorption and behavior. Langmuir compression and atomic force microscopy were used to examine the changes in microgel and monolayer characteristics during adsorption and equilibration.

Findings: Microgel interfacial adsorption was influenced by bulk concentration and particle size, with smaller particles adsorbing faster. Microgel-stabilized interfaces were dominantly elastic, and elasticity increased more rapidly when smaller microgels were employed as stabilizers. Interfacial compression increased surface pressure but not elasticity, possibly due to mechanical disruption of inter-particle interactions. Monolayer images showed the presence of small aggregates, suggesting that microgel structure can be disrupted at low interfacial loadings. The ability of β-lactoglobulin microgels to form highly elastic interfacial layers may enable improvements in the colloidal stability of food, pharmaceutical and cosmetic products in addition to applications in controlled release and flavor delivery systems.

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1. Introduction

Liquid emulsions are important for the structure and functionality of a wide variety of products, including beverages, pharmaceuticals and cosmetics. Liquid emulsions consist of droplets of one liquid phase dispersed within a second, immiscible liquid. The high surface energy of the liquid/liquid interface contributes to the instability of these emulsion products during storage. To create liquid emulsions with a finer structure and longer storage-life, amphiphilic molecules are adsorbed to the droplet interface, reducing surface energy and minimizing emulsion instability mechanisms, such as flocculation and coalescence. However, some of the most effective and widely surface-active amphiphilic molecules used to stabilize emulsions are perceived negatively by consumers who harbor a growing distrust of processed, artificial and unfamiliar components, particularly in foods [5,12]. In response, growing efforts have focused on the development and utilization of naturally-sourced emulsion stabilizers, including proteins [2].

Colloidal-scale particles can be used to stabilize emulsions provided that the particles are partially wetted by both the continuous and dispersed phases [24]. Particle-stabilized or Pickering emulsions exhibit extreme stability to droplet coalescence due to the formation of thick interfacial layers and the high energy required to desorb the particles from the interface [13]. For example, the energy to desorb a spherical particle from an oil–water interface (ΔG) can be calculated using the following equation [26]:

$$\Delta G = \pi r^2 \gamma_{ow}(1 - |\cos\theta|)^2 \quad (1)$$

where r is the radius of the particle, γ_{ow} is the oil–water interfacial tension, and θ is the three-phase contact angle of the particle between the solid and two liquids. As long as the contact angle is not close to 0° or 180° (indicating a lack of wettability by one of the phases), absorption of particles larger than 10 nm in radius may be considered practically irreversible, as the energy required to desorb a particle is greatly in excess of the thermal energy of the particle (>10 kT) [9]. Until recently, research into Pickering stabilizers has focused on rigid particles composed of inorganic compounds, particularly silica and metal oxides with various surface treatments [1], which are of limited value in food systems. However, growing attention has been directed toward the use of deformable biopolymer-based particles as emulsion stabilizers [17], including microgels derived from proteins.

Milk is an excellent source of nutritious and surface-active proteins, including caseins, serum albumins, immunoglobulins, β-lactoglobulin (Blg), and α-lactalbumin [28]. During cheese manufacturing, the liquid waste stream (whey) removed from the casein-rich curd is rich in Blg and α-lactalbumin, providing a source of inexpensive and readily available materials for developing novel food ingredients [4]. Blg is a relatively small food protein with a molecular weight of 18.2 kDa and a rich amino acid composition [16]. With an isoelectric point of approximately 4.8–5.2, Blg possesses a net-negative charge under neutral pH conditions and a net-positive charge under acidic conditions [16].

Microgel is a general term describing colloidal particles with an internal structure of cross-linked, yet still solvated, polymers [21]. Depending upon the relative solvation of the polymers and the degree of cross-linking, microgels are able to swell or shrink as the solvation quality increases or decreases, with the microgels highly deformable in the more swollen state [30]. Aggregation of whey proteins, particularly Blg, by thermal treatment under mildly acidic conditions has been found to produce colloidal particles of ~100–300 nm in size [11,15]. Scattering experiments demonstrated that these colloidal particles are microgels, where the hydrated proteins are cross-linked by disulfide- and hydrogen-bonds in a loose fractal network [27]. Interestingly, reducing agents did not significantly inhibit the formation of Blg microgels but rather increased their size at high molar ratios, indicating the dominant role of non-covalent interactions within the microgel network [19]. Blg microgel size can be further influenced by minute changes in the ionic strength [23] or fabrication pH, with larger particles forming at higher ionic strengths and lower pH values [19,22].

Given the lack of available literature regarding the adsorption and interfacial behavior of protein-based microgels, it is useful to consider synthetic systems, such as poly(N-isopropylacrylamide) (pNIPAM) microgels, as a model. Stabilization of fluid interfaces by pNIPAM microgels was shown to consist of two distinct processes: diffusion-limited adsorption to the interface and deformation of microgels on the interface [18]. Adsorption of such colloidal particles to the interface in relatively dilute conditions occurs by diffusion, which diminishes with greater microgel size [6]. Once adsorbed, deformation of the particles exposes more of the particle material to the interface and further decreases the surface energy [8]. Thus, reducing the deformability of microgel particles (e.g. greater internal cross-linking) reduces their stabilizing capabilities [18]. While recent studies have shown the capacity of microgels formed from Blg or whey protein isolate to stabilize oil-in-water emulsions [7,31], as well as water-in-water emulsions [20], there is little information on the interfacial behaviors of these microgels. Interfacial properties of Blg microgels are expected to be broadly similar to the pNIPAM microgels given their similar structural and surface active properties.

The objective of this work was to evaluate the interfacial properties of Blg-based microgels of varying sizes, with the hypothesis that smaller Blg microgels would adsorb to the interface more rapidly and lead to more elastic, tightly-packed interfaces. Particle size, controlled by fabrication pH, was evaluated using light scattering techniques and atomic force microscopy. Dynamic interfacial tension measurement was used to evaluate the spontaneous adsorption of microgels to the interface at varying bulk concentrations, while dilatational measurements were used to observe the viscoelastic properties of the interfaces. Behavior and packing of microgels at the interface were determined during compression at a planar air–water interface, with the morphology of microgels adsorbed to the interface observed by atomic force microscopy after extraction of the monolayer.

2. Materials and methods

2.1. Materials

β-Lactoglobulin (Blg, lot# JE 001-0-415), with a reported composition of 97.9% protein (91.5% Blg), 0.2% fat, 1.8% ash, and 4.4% moisture, was donated by Davisco Food International (Le Sueur, MN). Blg was further purified of non-native protein and ions using a previously published protocol [14]. Sodium hydroxide, sodium acetate, concentrated hydrochloric acid, n-heptane and Florisil (activated magnesium silicate, 100–200 mesh) were obtained from Sigma Chemical Co. (St. Louis, MO). All solutions were prepared using ultrapure water ($\sigma \geqslant 18$ mΩ cm) obtained from a filtration system (Barnstead E-pure, Thermo Scientific, Waltham, MA).

2.2. Methods

0.25% (w/w) Blg was mixed thoroughly in 2.5 mmol kg^{-1} $NaC_2H_3O_2$ buffer at pH 6.0 and acidified to pH 5.70 or 5.90 using 0.1 M HCl solution. Microgels were formed by submerging 16 × 100 mm glass tubes filled with 12 mL Blg solution in a hot-water bath at 85 °C for 20 min directly followed by submersion in an ice-water bath for 20 min. Samples were either analyzed on