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Characterization of β-lactoglobulin–sodium alginate interactions in aqueous solutions: A calorimetry, light scattering, electrophoretic mobility and solubility study

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Abstract

The influence of pH (3–7) on the properties of sodium alginate (NaA), β-lactoglobulin (β-Lg) and their mixtures in aqueous solutions was studied at 30 °C using isothermal titration calorimetry (ITC), dynamic light scattering, turbidity, ζ-potential and soluble protein measurements. The electrical charge of isolated β-Lg went from positive to negative as the pH increased from 3 to 7 with the isoelectric point being around pH 4.8. The electrical charge of isolated sodium alginate was negative at all pH values, but was appreciably lower in magnitude from pH 3 to 4 due to partial protonation of carboxyl groups ($pK_a \approx 3.5$). Light scattering measurements indicated that isolated sodium alginate was completely soluble from pH 3 to 7, but isolated β-Lg formed large complexes ($d_V > 200$ nm) that scattered light at pH 4 and 5. When β-Lg and sodium alginate were mixed together at pH 3 and 4 they formed large complexes ($d_V > 1000$ nm) due to electrostatic attraction between the oppositely charged molecules. At pH 5, β-Lg and sodium alginate formed fairly soluble complexes due to electrostatic attraction between the anionic polysaccharide and cationic patches on the protein surface. At pH 6 and 7, β-Lg and sodium alginate did not form complexes due to electrostatic repulsion between the similarly charged molecules. The knowledge gained in this study could be used to facilitate the rational design of food ingredients and products with desirable functional properties.

Keywords: β-lactoglobulin; Sodium alginate; Interaction

1. Introduction

Proteins and polysaccharides are used together as functional ingredients in many kinds of food products, including solutions, gels, emulsions and foams (Dickinson, 1992, 1993, 2003; Tolstoguzov, 1997, 2002, 2003a,b). The control or manipulation of the macromolecular interactions between these two different kinds of biopolymer is a key factor in the development of novel food processes and products as well as in the formulation of fabricated food products (Grinberg & Tolstoguzov, 1997; Tolstoguzov, 1997, 2003a,b; Weinbreck, de Vries, Schrooyen, & de Kruif, 2003). The functional properties of each kind of biopolymer are often modified by the presence of the other kind of biopolymer. For example, the solubility, conformational stability, gel-forming ability, surface activity, emulsifying properties, and foaming properties of many proteins are strongly affected by their interactions with polysaccharides. Conversely, the ability of many polysaccharides to thicken solutions, form gels or stabilize emulsions is influenced by the presence of proteins. An improved understanding of the molecular basis of protein–polysaccharide interactions and the impact of these interactions on the bulk physicochemical and sensory properties of foods would therefore enable food scientists to design foods in a more rational and systematic fashion.

Protein–polysaccharide interactions are particularly sensitive to the precise molecular characteristics of the protein and polysaccharide molecules involved, as well as to solution conditions that modulate the dominant intermolecular forces such as pH, ionic composition and temperature (Benichou, Aserin, & Garti, 2002; de Kruif & Tuinier, 2001; de Kruif, Weinbreck, & de Vries, 2004; Dickinson, 2003;

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McClements, 2004; Tolstoguzov, 1997). Aqueous solutions of mixed biopolymers are susceptible to phase separation by one of two alternative mechanisms—complex coacervation (associative phase separation) or thermodynamic incompatibility (segregative phase separation) (Dickinson & McClements, 1995; Tolstoguzov, 1997, 2003a,b). Complex coacervation occurs when two biopolymers are strongly attracted to each other, which usually occurs because they have opposite electrical charges. This leads to the formation of a two-phase system that consists of a mixed biopolymer complex phase suspended in a solvent phase depleted in both biopolymers. This complex may be either soluble or insoluble depending on the electrical characteristics of the biopolymers involved and the solution composition. On the other hand, thermodynamic incompatibility tends to occur when the biopolymers are uncharged or similarly charged so that there is a relatively strong steric or electrostatic repulsion between them. In this situation, the two biopolymers may co-exist in a single phase (miscibility) in domains in which they mutually exclude one another or, above a critical concentration, segregate into two different phases. Each of the phases formed is rich in one type of biopolymer and depleted in the other type.

In this study, we examined the influence of pH on the interaction between bovine β-lactoglobulin and sodium alginate in aqueous solutions. Bovine β-lactoglobulin is obtained from cow's milk, where it is the major protein in the whey fraction. Structurally, it is a compact globular protein (molecular mass = 18.3 kDa) containing 162 amino acid residues with one thiol group and two disulfide bonds. The isoelectric point of β-lactoglobulin has been reported to be around 4.7–5.2 (Bromley, Krebs, & Donald, 2005; Das & Kinsella, 1989; Sawyer & Kontopidis, 2000). The association properties of β-lactoglobulin in aqueous solution depend strongly on pH (Gottschalk, Nilsson, Roos, & Halle, 2003). At pH 5–8, β-lactoglobulin exists as a dimer, at pH 3–5 the dimers associate to form octomers, and at extreme pH values (<2 or >8) β-lactoglobulin exists mainly as monomers. At pH >9, the molecule is irreversibly denatured.

Alginates are salts of alginic acids (Morris, 1998). They occur naturally as the major structural polysaccharides of brown marine algae (*Phaeophyceae*) and as extracellular mucilages secreted by certain species of bacteria. They are linear polymers of (1→4)-β-D-mannuronopyranosyl and (1→4)-α-L-guluronopyranosyl units in a copolymer that contains homopolymeric sequences. The dissociation constants for mannuronic acid (M) and guluronic acid (G) monomers are 3.38 and 3.65, respectively (Draget, 2000). Hence, alginates tend to be negatively charged across a wide range of pH values. Sodium alginates are used as stabilizers, thickeners and gelling agents in several foods, such as sauces, soups, beverages and deserts (Whistler & BeMiller, 1997).

Previous studies have shown that globular proteins can interact with anionic polysaccharides (e.g. gum arabic, carboxymethycellulose, pectin, and carrageenan) to form either soluble or insoluble complexes (Chang, Lu, Chen, Tu, & Hwang, 2000; de Kruif et al., 2004; Ducel, Richard, Saulnier, Popineau, & Boury, 2004; Girard, Turgeon, & Gauthier, 2002, 2003; Weinbreck et al., 2003; Weinbreck, Nieuwenhuijse, Robijn, & de Kruif, 2004). These complexes may be stabilized predominantly by electrostatic, ion-dipole or hydrophobic interactions. An improved understanding of the origin and nature of these interactions would lead to the design of foods with improved nutritional, physicochemical, and sensory properties. Therefore, in this study we investigated the interactions between β-lactoglobulin and sodium alginate in aqueous solutions using a variety of complementary techniques: isothermal titration calorimetry (ITC), dynamic light scattering, turbidity, ζ-potential, and protein solubility measurements.

2. Materials and methods

2.1. Materials

Food grade sodium alginate was kindly donated by TIC gums (TIC Pretested® Colloid 488T, lot no. 6724). The supplier reported that this product had an M:G ratio of about 55:45, was of 'medium' viscosity, and had a molecular mass of 216 kDa. Powdered β-lactoglobulin (β-Lg) was obtained from Davisco Foods International (lot no. JE 001-3-922, Le Sueur, MN). The manufacturer reported that this product contained 95% β-Lg and 4.9% moisture. Analytical grade hydrochloric acid, sodium hydroxide, sodium azide, and sodium phosphate were obtained from Sigma-Aldrich (St. Louis, MO). Purified water from a Nanopure water system (Nanopure Infinity, Barnstead International, IA) was used for the preparation of all solutions.

2.2. Solution preparation

A stock buffer solution was prepared by dispersing 5 mM phosphate buffer in water containing 0.04 wt% NaN_3 (using as an antimicrobial) and then adjusting to pH 7 using HCl or NaOH. Biopolymer stock solutions were prepared by dissolving either 1.0 wt% β-Lg or 1.0 wt% sodium alginate in the stock buffer solution, stirring for at least 2 h to ensure complete dispersion, and then storing overnight at 5 °C. Biopolymer mixtures containing β-Lg (0.1 wt%) and sodium alginate (0–0.1 wt%) were prepared by mixing different ratios of the stock solutions with buffer solution at the desired pH (3–7). The pH of each of the solutions was adjusted to the appropriate value prior to mixing. It should be noted that there would be a slight difference in the ionic strength of solutions at different pH values due to the different amounts of acid or base they contained.

2.3. Isothermal titration calorimetry (ITC)

An isothermal titration calorimeter (VP-ITC, Microcal Inc., Northampton, MA) was used to measure the enthalpy