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Encapsulation of protein nanoparticles within alginate microparticles: Impact of pH and ionic strength on functional performance

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ABSTRACT

Incorporation of bioactive proteins into functional foods is often challenging due to their instability to aggregation, sedimentation, or hydrolysis. In this study, core–shell protein nanoparticles, consisting of a zein core and a whey protein shell, were fabricated by antisolvent precipitation. The protein nanoparticles were then incorporated into biopolymer microgels fabricated by electrostatic complexation of casein and alginate. Protein nanoparticles were retained in microgels at low pH (3–5.5), but released at higher pH (6–7) due to microgel dissociation promoted by electrostatic repulsion between anionic casein and alginate. These microgels may be useful for retaining and protecting protein nanoparticles within acidic environments (*e.g.*, stomach), but releasing them under neutral environments (*e.g.*, small intestine). Protein nanoparticles were retained within microgels over a wide range of ionic strengths (0–2 M NaCl, pH 5). Protein nanoparticle encapsulation within microgels may improve their pH and salt stability in functional foods.

1. Introduction

There is growing interest in the utilization of proteins and peptides (referred to collectively as "polypeptides" for convenience) as functional ingredients in foods because of their beneficial health effects, such as antioxidant, antimicrobial, and antihypertension activities (Hernandez-Ledesma et al., 2011; Samaranayaka and Li-Chan, 2011; Sarmadi and Ismail, 2010; Udenigwe and Aluko, 2012). Typically, the activity of polypeptides depends on their three-dimensional structures and specific amino acid sequences (Udenigwe and Aluko, 2012). Polypeptide activity may therefore be altered in food products during manufacture, storage, or transportation, due to changes in solution or environmental conditions that alter protein structure, such as pH, ionic strength, ingredient interactions, or temperature (Hettiarachchy et al., 2012). In addition, their activity may be altered after they are ingested and pass through the gastrointestinal tract (GIT) because they are exposed to digestive enzymes (proteases and peptidases) and environmental conditions (pH, ionic strength, and ingredient interactions) that may alter their structure (Mohan et al., 2015). Within the GIT, digestive enzymes and highly acidic gastric conditions may hydrolyze polypeptide chains at particular bond locations, thereby generating new peptides (Moreno, 2007). This process may be undesirable if it leads to loss of the bioactivity of an ingested polypeptide, or it may be desirable if it leads to the generation of new peptides with improved bioactivity. Consequently, it is often important to design food matrices that can control the gastrointestinal fate of polypeptides within foods and within the GIT so as to improve their bioactivity profiles (Mohan et al., 2015; Zhang et al., 2015b).

The encapsulation of polypeptides within colloidal delivery systems offers a potentially effective means of controlling their stability both in food products and within the GIT after ingestion (Mohan et al., 2015; Sagalowicz and Leser, 2010; Zhang et al., 2015b). For food applications, polypeptides are typically trapped within a colloidal particle that is fabricated from food-grade

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ingredients, such as lipids, carbohydrates, proteins, surfactants, or minerals (McClements, 2014). Polypeptides may be hydrophilic, hydrophobic, or amphiphilic depending on their amino acid composition and structural organization (Udenigwe and Aluko, 2012). Consequently, an appropriate encapsulation material and structure must be selected for the particular polypeptide involved. Numerous kinds of colloidal delivery systems have been explored for their potential to encapsulate polypeptides and other bioactive components, including microemulsions, nanoemulsions, emulsions, solid lipid nanoparticles, multiple emulsions, biopolymer particles, and microgels (Du and Stenzel, 2014; du Plessis et al., 2014; McClements, 2014; Mohan et al., 2015). Each of these delivery systems has advantages and disadvantages in terms of its ease of preparation, storage, and handling, cost, stability characteristics, encapsulation efficiency, loading capacity, and food matrix compatibility.

Previously, complex coacervation has been used by our group to develop hydrogel particles containing encapsulated lipid droplets (Li and McClements, 2011; Zhang et al., 2015a, 2015b). These filled hydrogel particles have a number of potential applications in the food industry, including improving physical or chemical stability, targeted delivery, and regulation of lipid digestion and satiety (Zhang et al., 2015c). In the current study, we investigated the possibility of encapsulating protein nanoparticles within polysaccharide-based hydrogel particles ("microgels"), and studied the influence of solution and environmental conditions on their properties. The main focus of this study was to examine the influence of solution conditions (pH and ionic strength) on the stability and properties of the protein nanoparticle-loaded microgels. This information is useful for establishing their potential application in food products, and their potential behavior within the gastrointestinal tract.

2. Materials and methods

2.1. Materials

Zein (Lot# SLBD5665V) and sodium alginate (Lot 50K0180) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Sodium caseinate powder was obtained from the American Casein Company (MP Biomedicals LLC). Whey protein isolate (WPI) powder was obtained from Davisco Foods International Inc. (Le Sueur, MN, USA). Alginic acid (sodium salt) (Lot# 180947) was purchased from the Sigma Chemical Company (St. Louis, MO). All other chemicals were of analytical grade. Double distilled water was used to prepare all solutions and colloidal suspensions.

2.2. Solution preparation

A weighed amount (2.64 g) of zein powder was added to 100 mL ethanol solution (80% *v/v*), and stirred at 500 rpm (IKA R05, Werke, GmbH) for 1 h, and then filtered with filter paper (Fisher Science, P5). WPI (0.25% *w/v*) was solubilized in phosphate buffer solution (10 mM, pH 6.5). 2% (*w/w*) sodium caseinate and 2% (*w/w*) alginate were prepared separately in 10 mM phosphate buffer at pH 7 and stirred until fully dissolved.

2.3. Fabrication of colloidal particles

2.3.1. Protein nanoparticles preparation

Protein nanoparticles were fabricated from zein using an antisolvent precipitation method. Initially, zein (26.4 mg/mL) was dissolved in ethanol solution (80% *v/v*). Then, 25 mL of aqueous ethanol solution was rapidly injected into 75 mL of whey protein solution (0.25% WPI, PBS, pH 6.5) that was continuously stirred at 1200 rpm using a magnetic stirrer (IKA R05, Werke, GmbH). The resulting colloidal dispersion was then stirred for another 30 min at the same speed. The ethanol remaining in the final colloidal dispersions (around 16% *v/v*) was evaporated at 40 °C using a rotary evaporator (Rotavapor R110, Büchi Corp., Switzerland), and the same volume of pH 6.5 PBS was added to compensate for the lost ethanol.

2.3.2. Unfilled hydrogel particle preparation

2 M sodium hydroxide was used to adjust caseinate (2%) and alginate (2%) solutions to pH 7. Then these two stock solutions and phosphate buffer were mixed together at different volume ratios under continuous stirring to form final compositions of 0.33% sodium caseinate/1.33% alginate (mass ratio 1:4). The mixtures were then acidified to pH 5 using 1 M citric acid at a rate of 1 drop/10 s with continuous stirring at 500 rpm to promote complex formation.

2.3.3. Filled hydrogel particle preparation

2 M sodium hydroxide was used to adjust caseinate solutions, alginate solutions, and protein nanoparticle dispersions to pH 7. After pH adjustment, 6.6 mg/mL protein nanoparticle dispersion and 2% sodium caseinate solution were mixed together at a 1:1 volume ratio. Then, this system was mixed (500 rpm) with 2% alginate solution at a volume ratio of 1:2. Finally, the resulting mixture was acidified to pH 5 using 1 M citric acid with continuous stirring at 500 rpm to promote complex formation. The final composition of the resulting system was 1.1 mg/mL protein nanoparticles, 0.33% sodium caseinate, and 1.33% alginate.

2.4. ζ-potential measurements

The electrical charge (ζ-potential) of biopolymers and colloidal particles was measured at different pH values (3.0–7.0) using a particle electrophoresis instrument (Zetasizer Nano ZA series, Malvern Instruments Ltd. Worcestershire, UK). Samples were diluted using 10 mM phosphate buffer (at the same pH as the sample) prior to analysis to avoid multiple scattering effects. All measurements were made on at least two freshly prepared samples and each sample was measured in duplicate.

2.5. Particle size analysis

The particle size distribution was measured using a static light scattering instrument (Mastersizer 2000, Malvern Instruments, Worcestershire, United Kingdom). This instrument infers the size of the particles from measurements of their angular scattering pattern. All measurements were made on at least two freshly prepared samples. Samples were diluted in 10 mM phosphate buffer (pH 3.0–7.0) by adding small aliquots into a measurement chamber. Particle size measurements were reported as surface-weighted mean diameters (d_{32}).

2.6. Microstructural analysis

The microstructure of the colloidal dispersions was characterized using confocal scanning fluorescence microscopy (Nikon D-Eclipse C1 80i, Nikon, Melville, NY, U.S.). Prior to analysis, the protein phase of the samples was dyed with fluorescein thiocyanate isomer I (FITC) solution (1 mg/mL dimethyl sulfoxide) by adding 0.1 mL of FITC dye solution to 2 mL of sample. All images were captured with a 10 × eyepiece and a 60 × objective lens (oil immersion). The microstructure images for confocal microscopy were digitally acquired and then analyzed using image analysis software (NIS-Elements, Nikon, Melville, NY).