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Control of protein digestion under simulated gastrointestinal conditions using biopolymer microgels

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ABSTRACT

Controlling the rate and extent of protein digestion within different regions of the human gastrointestinal tract (GIT) is important for regulating hormonal responses (such as hunger, satiety, and satiation) and immune responses (such as allergenicity). In this study, hydrogel beads (biopolymer microgels) were fabricated by injecting a solution of anionic alginate molecules into a solution of cationic calcium ions using a vibrating extrusion device. A model food protein (whey protein isolate, WPI) was mixed with the alginate solution prior to bead formation. The impact of thermal processing (80 °C, 15 min) of the WPI before or after microgel formation was examined to determine the impact of protein denaturation and aggregation on encapsulation efficiency and retention. Heat-denaturation of the protein prior to microgel formation led to the highest encapsulation efficiency and retention, which was attributed to the formation of a cold-set protein gel inside the beads. Simulated GIT studies indicated that protein encapsulation in the microgels retarded its digestion in the stomach (around 3.7% digested), but not in the small intestine (around 19.6% digested). The denatured and native proteins were digested differently in different GIT regions: denatured protein digested faster in the stomach (around 11% digested), whereas native protein digested faster in the small intestine (around 41% digested). These results could provide valuable information for the design of microgel-based delivery systems to regulate protein digestion and peptide release in the GIT.

1. Introduction

Proteins play a number of important roles as nutritional components within the human diet. They are a source of essential amino acids and bioactive peptides, provide energy, are potential allergens, and regulate hormonal responses, such as hunger, satiety and satiation (Bendtsen, Lorenzen, Bendsen, Rasmussen, & Astrup, 2013; Dangin et al., 2003; Erdmann, Cheung, & Schroder, 2008; Halford & Harrold, 2012; Moreno, 2007; Samaranayaka & Li-Chan, 2011). The rate, extent, and location of protein hydrolysis within the human gastrointestinal tract (GIT) impact many of these attributes. Protein hydrolysis is typically carried out by digestive enzymes within the stomach and small intestine (Moreno, 2007; Picariello, Mamone, Nitride, Addeo, & Ferranti, 2013). In humans, protein digestion begins in the stomach due to the presence of gastric pepsins that are activated under acidic conditions (Freeman & Kim, 1978). These enzymes breakdown peptide bonds within the protein molecule, which results in the formation of a mixture of polypeptides, oligopeptides, and amino acids. After leaving the stomach, any remaining proteins or peptides are further hydrolyzed by pancreatic enzymes (such as trypsin, chymotrypsin, elastase and carboxypeptidases A and B) activated in the duodenum (Erickson & Kim, 1990). Eventually, the amino acids and small peptides resulting from protein digestion are absorbed by the epithelium cells and enter the systemic circulation. The products of protein digestion impact various human functions regulated by hormones, including gastrointestinal motility, gastric emptying time, secretion of acid and pepsinogen, and total protein and energy intake (Caron, Domenger, Dhulster, Ravallec, & Cudennec, 2017; Sah, McAinch, & Vasiljevic, 2016). It may therefore be possible to modulate these hormonal responses by controlling the rate and extent of protein digestion within the GIT.

Structural design of food matrices has been proposed as a method of controlling the digestion and release of macronutrients within the GIT, and therefore as a means to modulate their physiological effects (Norton, Espinosa, Watson, Spyropoulos, & Norton, 2015; Sah et al., 2016). In the current study, we examined the impact of encapsulating proteins inside biopolymer microgels on their gastrointestinal fate using an *in vitro* GIT model. Our previous studies showed that encapsulation of lipids inside biopolymer microgels retarded the rate and extent of lipid digestion under simulated GIT conditions, which was attributed to

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the ability of the biopolymer network to restrict interactions between the digestive enzymes and their substrates (Samaranayaka & Li-Chan, 2011; R. Zhang et al., 2016). Biopolymer microgels are normally fabricated from food-grade proteins and/or polysaccharides (Chen, Remondetto, & Subirade, 2006; Zhang, Zhang, Chen, Tong, & McClements, 2015). The substance to be encapsulated is typically mixed with a biopolymer solution, which is then gelled by adding an appropriate cross-linking agent (such as an ion, acid, base or enzyme) or by changing environmental conditions (such as temperature or pressure) (Shewan & Stokes, 2013; Z. Zhang et al., 2015). Numerous approaches can be used to fabricate protein-loaded microgels, including injection, templating, and phase separation methods (McClements, 2017a, 2017b). The injection-gelation method is one of the most commonly utilized approaches for encapsulating bioactive substances in biopolymer microgels because of its relative simplicity and low cost. In this approach, an aqueous solution containing the bioactive agent and a gelling biopolymer is injected into a "hardening" solution to promote particle formation and biopolymer cross-linking. If carried out correctly, this procedure results in the formation of biopolymer microgels with bioactive agents trapped inside. The GIT fate of the bioactive agent can be modulated by changing the composition, dimensions, or pore size of the microgels (Z. Zhang et al., 2015).

In the current study, we examined the possibility to control the digestion of proteins under simulated GIT conditions by encapsulating them inside biopolymer microgels fabricated from calcium alginate. Our previous studies have shown that native proteins can easily escape from biopolymer microgels under conditions where there is not a strong attraction between the biopolymer network and protein molecules, *i.e.*, at pH values above the isoelectric point of the protein (Zhang, Zhang, Zou, & McClements, 2016). This phenomenon was mainly attributed to the fact that the pore size of the microgels is appreciably greater than the dimensions of the native protein molecules. Consequently, the protein molecules can easily diffuse out of the microgels when there is no electrostatic attraction between them and the biopolymer network. For this reason, we examined the impact of using a thermal treatment to promote protein denaturation and aggregation inside the microgels on protein encapsulation and retention. The resulting protein-loaded microgels were passed through a simulated GIT that included mouth, stomach, and small intestinal phases. An *in vitro* pH-stat method was used to determine the degree of protein hydrolysis in the simulated stomach and small intestine phases. We hypothesized that the encapsulation of denatured protein inside the microgels would retard its digestion and release under simulated GIT conditions. The information obtained in this study may be useful for the development of functional foods to regulate gastrointestinal responses, such as hunger, satiety, and satiation.

2. Materials and methods

2.1. Materials

Whey protein isolate (WPI) was kindly provided by Davisco Foods International Inc. (Le Sueur MN). The following chemicals were purchased from the Sigma Chemical Company (St. Louis, MO): alginic acid (sodium salt); pepsin from porcine gastric mucosa; fluorescein isothiocynate (FITC) isomer I; calcium chloride dehydrate; porcine trypsin and bovine α-chymotrypsin. All chemicals used were analytical grade. Double distilled water was used to prepare all solutions. All concentrations are expressed as weight percentages (% w/w), unless otherwise stated.

2.2. Heated protein preparation

Protein solutions (10% WPI) were prepared by dissolving powdered whey protein isolate into phosphate buffer solution at pH 7 with continual stirring. In some cases, the dissolved protein solutions were heated at 80 °C for 15 min in a water-bath, then rapidly transferred to an ice-water bath for 10 min, and then stored at room temperature for 24 h.

2.3. Protein-loaded microgel preparation

For the pre-loading denatured protein microgels (pre-D-MGs), an aqueous alginate solution was first prepared by dissolving powdered sodium alginate (1.6%) in phosphate buffer with continual stirring until complete dissolution. The heated WPI and alginate solutions were then mixed together (1:1, v/v) for 1 h with continual stirring to form a uniform solution. The resulting mixture was then injected into a 10% calcium chloride solution using a commercial encapsulation unit with a 120 μm vibrating nozzle (Encapsulator B-390, BUCHI, Switzerland). For the protein-free microgel preparation, just alginate solutions were injected into calcium chloride solution using the same procedure (no protein). The microgels formed by this process were then kept in the calcium chloride solution for 1 h at ambient temperature to promote cross-linking of the alginate molecules. For the digestion experiments, the calcium alginate beads were collected by filtration and then washed with phosphate buffer and distilled water to remove any excess ions from their surfaces. After determining the total weight of the dry beads, they were stored in a refrigerator for further measurements.

Two control samples were also fabricated to determine the impact of protein state on protein encapsulation and retention: native WPI-loaded microgels (N-MGs) and post-loading denatured protein microgels (post-D-MGs). The N-MGs were fabricated by encapsulation of unheated whey protein (rather than denatured protein) inside the microgels using the same procedure as described above. The post-D-MGs were prepared by firstly fabricating the native WPI-loaded microgels, and then heating them at 80 °C for 15 min in a water-bath. The resulting microgels were then cooled using an ice-water bath for 10 min, and then stored at room temperature for 24 h.

2.4. Determination of protein content in microgels (pre-D-MGs)

After the formation of microgels in ion solutions, the heated WPI-loaded microgels were collected by filtration and then washed with phosphate buffer solution and distilled water to remove any excess calcium ions and free protein from their surfaces. The protein content in the calcium ion solution after the microgels had been removed was determined by measuring the absorbance at 280 nm using a UV–visible spectrophotometer. The protein concentration in the calcium ion solution was $< 6\%$, which meant that the majority of protein ($> 94\%$) had been successfully trapped inside the microgels during the fabrication process. The washed microgels were then incubated in phosphate buffer solution at pH 7 for 6 h. The protein content in the incubation phase was determined periodically using UV–visible spectrophotometry. (Ultrospec 3000 pro, Biochrom Ltd., Cambridge, UK). The results indicated $< 2\%$ of the protein was released from the microgels at the end of 6 hour incubation ($> 98\%$ retention). Consequently, we can conclude that the majority of the protein added to the initial protein-alginate solution used to fabricate the microgels ended up inside them. The amount of WPI lost during the fabrication of the beads (around 8%) was taken into account when carrying out the protein quantification experiments using the pH-stat in the following section.

2.5. Gastrointestinal tract model

For the simulated GIT studies, four samples were prepared using the procedures described in the previous sections: native WPI; heated WPI; heated WPI-loaded beads (pre-D-MGs); and, heated WPI mixed with beads. The heated WPI mixed with beads were prepared by mixing a heated WPI solution with un-loaded microgels. All the samples were then diluted with buffer solution (5 mM phosphate buffer, pH 7) to obtain the same initial protein amount (0.375 g). The diluted samples