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Modulating lipid droplet intestinal lipolysis by electrostatic complexation with anionic polysaccharides: Influence of cosurfactants

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ABSTRACT

The food, supplement, and pharmaceutical industries are interested in developing delivery systems that can control the biological fate of ingested lipids within the gastrointestinal tract. In this study, a simulated intestinal lipolysis model was used to elucidate the impact of cosurfactants and anionic polysaccharides on the digestion of emulsified fats. Lipid droplets were prepared by membrane homogenization using a globular protein (β-lactoglobulin) as the primary surfactant, and a non-ionic surfactant (Tween 20) as the cosurfactant. Electrostatic complexes were formed by mixing the resulting cationic lipid droplets with anionic alginate molecules. In the absence of cosurfactant, multilayer emulsions were formed consisting of lipid droplets coated by a layer of alginate. In the presence of cosurfactant, microclusters were formed that contained aggregates of alginate-coated lipid droplets linked together. The electrical charge on the complexes remained negative from pH 2 to 7.5, with the complexes formed in the presence of cosurfactant having a lower charge magnitude. The rate and extent of lipid digestion under simulated intestinal lipolysis conditions depended on cosurfactant, alginate, and digestion conditions (fasted *versus* fed). Under high calcium fed conditions (20 mM Ca^{2+}), lipid digestion was highly suppressed in delivery systems containing alginate but no cosurfactant, which was attributed to the formation of a calcium alginate gel that restricted access of lipase to the lipid droplets. This reduction in lipid digestion could be largely overcome by including cosurfactant in the delivery systems. The information obtained in this study may prove useful for designing oral delivery systems that control the digestion and release of lipids in the gastrointestinal tract.

1. Introduction

There is an increasing need for oral delivery systems for lipophilic bioactive compounds in the pharmaceutical, supplement, and food industries (McClements, Decker, Park, & Weiss, 2009; Manzano, Colilla, & Vallet-Regi, 2009; Matsusaki & Akashi, 2009; Pathania, Millard, & Neamati, 2009; Shi, 2009; Shi, Porter, Merdan, & Li, 2009; Singh & Dash, 2009). These delivery systems are needed to encapsulate and protect bioactive compounds during storage, but release them after ingestion at the appropriate location within the gastrointestinal tract. A variety of lipid-based delivery systems have been investigated for their potential in encapsulating, protecting, and releasing lipophilic bioactive components, including oil solutions, microemulsions, liposomes, suspensions, and emulsions (Gershkovich, Wasan, & Barta, 2008; Han et al., 2009; Lee et al., 2008; Nordly, Madsen, Nielsen, & Foged, 2009; Semalty, Semalty, Rawat, Singh, & Rawat, 2009). Emulsions are particularly suitable for many commercial applications because of their ease of production, high loading capacity, and ability to be incorporated into a variety of aqueous based products.

Recently, a number of structural design approaches have been developed to extend the functional performance of emulsion-based delivery systems (Eric Dickinson, 2013; Li, Kim, Park, & McClements, 2012; McClements & Li, 2010). Lipid droplets can be coated by layers of biopolymers using an electrostatic layer-by-layer deposition method to form *multilayer emulsions* (Caruso & Mohwald, 1999; Decher & Schlenoff, 2003; Guzey & McClements, 2006a). These biopolymer coatings can be designed to improve the stability of the encapsulated lipid droplets to environmental stresses (Aoki, Decker, & McClements, 2005; Gu, Regnier, & McClements, 2005; Guzey & McClements, 2006b; Ogawa, Decker, & McClements, 2003b, 2003a), to protect encapsulated bioactive components from chemical degradation (Djordjevic, Cercaci,

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Alamed, McClements, & Decker, 2007; Klinkesorn, Sophanodora, Chinachoti, McClements, & Decker, 2005), and to release encapsulated bioactive components in response to specific environmental triggers (Decher et al., 2003). An alternative structural design approach is to embed lipid droplets within hydrogel matrices, which can be formed using various methods, such as extrusion, templating, coacervation, and thermodynamic incompatibility (McClements & Li, 2010; Matalanis, Jones, & McClements, 2011). The dimensions, permeability, and environmental responsiveness of the hydrogel matrices can be controlled so as to alter the digestion and release of emulsified lipids within gastrointestinal conditions (Li & McClements, 2011; Li, Hu, & McClements, 2011). A potential benefit of using structured emulsions as delivery systems is that they can be fabricated entirely from natural food-grade (GRAS) ingredients (*e.g.,* lipids, proteins, and polysaccharides) using simple processing operations (*e.g.,* homogenization and mixing).

Lipid droplets are one of the most important building blocks used to assemble structured emulsions. These droplets are usually fabricated by homogenizing an oil and phase together in the presence of a hydrophilic emulsifier (McClements, 2012). A number of different homogenization devices can be used to form emulsions industrially, including high-shear mixers, colloid mills, sonicators, high pressure valve homogenizers, microfluidizers, and membrane devices (Walstra, 1993, 2003). The type of homogenizer used determines the characteristic of the lipid droplets produced, *e.g.,* the particle size distribution. Membrane devices are a particularly promising means of preparing lipid droplets for assembling structured emulsions because of the narrow particle size distributions produced, the mild processing conditions, the low energy consumption, the simplicity of operation, and the possibility for large scale production (Liu, Yang, & Winston Ho, 2011; Vladisavljevic, Kobayashi, & Nakajima, 2012). In this study, premix membrane emulsification was used to produce positively charged protein-coated lipid droplets, which were then mixed with negatively charged polysaccharides to create electrostatic complexes. Previously, we have shown that multilayer emulsions can be formed by coating anionic lipid droplets formed by membrane homogenization with cationic biopolymers, such as chitosan (Gudipati, Sandra, McClements, & Decker, 2010; Vladisavljevic & McClements, 2010). The presence of the biopolymer coating was found to alter the physical properties, chemical stability, and biological fate of the encapsulated lipids (Tokle, Lesmes, Decker, & McClements, 2012). The behavior of emulsions during human consumption is getting more and more attractive. Protein is a kind of common emulsifier, which is sensitive to the environmental conditions. Especially, the effect of physic-chemical environments during gastrointestinal tract on the proteolysis and structure change of protein has been studied (Maldonado-Valderrama, Terriza, Torcello-Gomez, & Cabrerizo-Vilchez, 2013; Mandalari, Mackie, Rigby, Wickham, & Mills, 2009; Singh & Sarkar, 2011). The protein can be broken down by proteases (pepsin, trypsin and chymotrypsin), which can also be inhibited by adding some components. The proteolysis of protein layer in the emulsion would affect the lipid digestibility. The interaction between surfactant and protein molecules in bulk solutions and at interfaces during lipid digestion have been reviewed (Wilde & Chu, 2011). Different protein interfacial coating had similar extent of lipid hydrolysis, which indicated proteins could not comprise a barrier to lipolysis (Maldonado-Valderrama et al., 2013).

In the present study, we used a simulated intestinal lipolysis model to study the influence of a non-ionic cosurfactant (Tween 20) on the behavior of electrostatic complexes formed from protein-stabilized lipid droplets and alginate. We hypothesized that the addition of the cosurfactant would alter the performance of the electrostatic complexes by altering their structural organization and physicochemical properties. Modulating the properties of electrostatic complexes by cosurfactant addition may prove to be a useful means of designing structured emulsions for encapsulation and delivery of bioactive lipid components in the food, supplement, and pharmaceutical industries. To the authors' knowledge, previous studies have not examined the influence of cosurfactants on the behavior of electrostatic complexes under simulated gastrointestinal conditions. The initial electrostatic complexes consisted of either fat droplets coated by alginate (multilayer emulsions) or flocculated fat droplets held together by alginate (microclusters). We hypothesized that the initial structural organization of the fat droplets in the electrostatic complexes may alter their subsequent behavior in the intestinal lipolysis model.

2. Materials and methods

2.1. Materials

Powdered β-lactoglobulin (BLG) was obtained from Davisco Foods International (Lot # JE 002-8-415, Le Sueur, MN), USA. Tween 20 (T20) was purchased from MP Biomedicals LLC. (Lot# 1131K), USA. Corn oil was purchased from a local supermarket and used without further purification. Alginic acid (sodium salt) (Lot# 180947, viscosity of 1% alginic acid in water is 15–20 cp) was purchased from Sigma–Aldrich (St. Louis, MO). Lipase from porcine pancreas, Type II (L3126, triacylglycerol hydrolase E.C. 3.1.1.3, PPL), and bile extract (porcine, B8613) were purchased from Sigma–Aldrich. Calcium chloride ($CaCl_2.2H_2O$) and sodium chloride (NaCl) were obtained from Fisher Scientific. Analytical grade hydrochloric acid (HCl) and sodium hydroxide (NaOH) were purchased from Sigma–Aldrich. Purified water from a Nanopure water system (Nanopure Infinity, Barnstead International, Dubuque, IA) was used for the preparation of all solutions.

2.2. Preparation of electrostatic complexes

Lipid droplets: The initial lipid droplets used to form the electrostatic complexes were prepared by membrane homogenization. The initial systems consisted of 20 wt% oil phase (corn oil) and 80 wt% aqueous phase (surfactant solution). The aqueous surfactant solutions contained either 5% BLG or 2% BLG/1% T20 dissolved in 5 mM phosphate buffer (pH 7.0). A coarse emulsion premix was initially formed by pouring the oil phase into the aqueous phase with continuous stirring. This mixture was then passed through a membrane homogenizer (MG-20-5, Kiyomoto Iron Works, Ltd., Japan). The input container was filled with 100–200 g of emulsion premix, and then compressed air was used to generate the pressure that forced this mixture through the membrane. An SPG membrane with 4.0 μm pore size, 8.5 mm inner diameter, and 0.8 mm wall thickness was supplied by SPG Technology Co., Ltd. (Sadowara, Japan).

Electrostatic complexes: Electrostatic complexes were formed by mixing emulsions containing cationic lipid droplets (stabilized by BLG or BLG/T20) with aqueous solutions containing anionic alginate molecules at pH 7.0, and then adjusting to pH 3.5 to promote lipid droplet–alginate interactions. The final composition of the systems was 1.0 wt% oil and 0.2 wt% alginate, with either 0.25% BLG (no cosurfactant) or 0.1% BLG/0.05% T20 (with cosurfactant).

2.3. Influence of pH on electrostatic complex stability

The aqueous suspensions containing the electrostatic complexes were diluted with 5 mM buffer solutions to the same final oil concentration, and then the pH was adjusted from 2.0 to 7.5. The

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