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Interaction of a bile salt (sodium taurocholate) with cationic (ε-polylysine) and anionic (pectin) biopolymers under simulated gastrointestinal conditions

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ABSTRACT

Dietary fibers are known to have beneficial effects on human health, which is partially attributed to their interactions with components in the gastrointestinal tract. In this study, the interaction of model bile salts with anionic (pectin) and cationic (polylysine) food-grade biopolymers, and their electrostatic complex, was examined. Isothermal titration calorimetry and titration measurements were used to characterize the nature of the interactions between the biopolymers and bile salts. Analysis of isothermal titration calorimetry data showed a marked difference for the taurocholate-pectin and taurocholate-ε-polylysine complexes, suggesting that sodium taurocholate binds strongly to ε-polylysine, presumably due to the attraction of oppositely electrical charges. While, the interaction between sodium taurocholate and pectin seems to be dominated by hydrophobic forces. On the other hand, the interaction of sodium taurocholate with a mixture of the two biopolymers showed a behavior that combined the characteristics of the individual systems. These results may have important implications for the design of functional foods to improve human health and wellness.

1. Introduction

Bile acids are a group of amphiphilic molecules naturally secreted by the human body that play an important role in the digestion, transport, and absorption of ingested lipids within the gastrointestinal tract (GIT) (Dawson & Karpen, 2015; Euston, 2017; Marin, Macias, Briz, Banales, & Monte, 2016). These molecules have a plate-like structure with a polar side and a non-polar side, which enables them to adsorb to oil-water interfaces (Cabral & Small, 2010). The surface activity of bile acids is important in lipid digestion for a number of reasons. First, the bile acids may adsorb to the surfaces of large lipid droplets in the GIT, thereby facilitating their further breakdown by reducing the interfacial tension and preventing their coalescence by forming a protective layer (Thomas, Holm, Rades, & Mullertz, 2012). The decrease in droplet size, leads to an increase in surface area available for lipase molecules to attach to, thereby increasing lipase activity (Euston, 2017). Second, the bile acids facilitate the adsorption of lipase molecules by displacing any other surface-active molecules (such as food emulsifiers) from the lipid droplet surfaces (Wilde & Chu, 2011). Third, the bile salts are an integral part of the mixed micelles that solubilize and transport lipid digestion products and bioactive lipids to the epithelium cells (Garidel, Hildebrand, Neubert, & Blume, 2000). Compositionally, the mixed micelles consist of lipid digestion products (monoacylglycerides and free fatty acids) and secreted biosurfactants (bile acids, cholesterol and phospholipids) (Maldonado-Valderrama, Wilde, Macierzanka, & Mackie, 2011). Structurally, they consist of micelles and vesicles that have hydrophobic domains capable of solubilizing lipophilic molecules, and then transporting them across the mucus layer to the epithelium cells where they can be absorbed (Yao, Xiao, & McClements, 2014). The fact that bile acids play such key roles in lipid digestion, transport, and absorption means that it is important to understand their behavior within the GIT and to determine the factors that alter their activity.

Food-grade biopolymers are known to interact with bile acids in the GIT, and thereby interfere with the normal roles they play during lipid digestion (Capuano, 2017; Cervantes-Paz et al., 2017; Pilosof, 2017). Bile acid/biopolymer interactions can alter the bioavailability of ingested lipids by altering their bioaccessibility, absorption, or transformation (Lairon, Play, & Jourdheuil-Rahmani, 2007; D. J.; McClements,

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Li, & Xiao, 2015). For instance, if bile acids strongly bind to biopolymers, then they may be unable to facilitate the formation and stabilization of lipid droplets, the displacement of other emulsifiers, or the solubilization and transport of lipid digestion products in the GIT. Biopolymers differ in their molecular, physical, and physiological properties (Capuano, 2017; Cui, 2005; Stephen, Phillips, & Williams, 2006). Molecularly, they vary in the number, type, sequence and bonding of the monomers, which determines their molar mass, branching, conformation, rigidity, hydrophobicity, and electrical characteristics. Physically, they vary in their solubility, thickening, gelling, and binding properties. Physiologically, they vary in their digestibility, as well as in their biological effects. Understanding the impact of specific biopolymer features on the GIT fate of lipids is therefore important. In this study, the role of the electrical properties of biopolymers was investigated by characterizing the interaction of bile acids with an anionic biopolymer (pectin), a cationic biopolymer (polylysine), and their electrostatic complex.

Pectin is commonly utilized by the food industry as a stabilizer or texture modifier in food and beverage products (Maxwell, N. JWaldron, & Morris, 2012; Thakur, Singh, Handa, & Rao, 1997), as well as to fabricate hydrogel-based delivery systems (Munarin, Tanzi, & Petrini, 2012). The pectin molecule consists of linear anionic regions consisting of $\alpha_{1\text{-}4}$-linked D-galacturonic acid (GalA) monomers, as well as branched regions comprised of various kinds of neutral monosaccharides (mainly rhamnose, xylose, mannose, and arabinose). The GalA units have carboxyl groups, which may be free or methyl-esterified, depending on the origin, isolation, and processing of the specific type of pectin used (Thakur et al., 1997; Yapo, 2011). Pectin has been shown to reduce the rate and extent of lipid digestion using simulated GIT studies, which was mainly attributed to its ability to interact with various food and gastrointestinal components (Beysseriat, Decker, & McClements, 2006; Espinal-Ruiz, Parada-Alfonso, Restrepo-Sánchez, Narváez-Cuenca, & McClements, 2014; Gunness & Gidley, 2010). The ability of pectin to bind bile acids reduces the level of surface-active components available to form/stabilize lipid droplets, alter interfacial composition, and solubilize/transport lipid digestion products (Hur, Lim, Decker, & McClements, 2011; McClements & Li, 2010). The binding of bile acids by pectin has been attributed to its ability to reduce blood cholesterol levels, as binding inhibits their re-absorption by the body (Pfeffer, Doner, Hoagland, & McDonald, 1981).

Foods may also contain cationic biopolymers that can interact with bile acids and thereby interfere with their normal function during lipid digestion. The polypeptide ε-polylysine (ε-PL) is a natural cationic homopolymer consisting of L-lysine monomers (typically between 25 and 35) linked together by isopeptide bonds between ε-amino and α-carboxyl groups (Yamanaka et al., 2010; Yoshida & Nagasawa, 2003). ε-Polylysine has been shown to have strong antimicrobial activity against various types of bacteria, yeasts, molds, and bacteriophages (El-Sersy, Abdelwahab, Abouelkhiir, Abou-Zeid, & Sabry, 2012; Hyldgaard et al., 2014; Moschonas, Geornaras, Stopforth, Wach, Woerner, Belk, et al., 2012; Yu, Huang, & Huang, 2010; Zhou, Li, Qi, Sharif, Poon, Cao, et al., 2011). Consequently, it can be used to control microbial growth in foods and beverages due to its strong antimicrobial activity, low toxicity, high water-solubility, and good thermal stability (Yoshida & Nagasawa, 2003). The presence of primary amine groups along the backbone of ε-PL means that it has a relatively high isoelectric point (pI ≈ 9), and so it tends to be strongly positively charged at the pH values found in most foods (Yoshida & Nagasawa, 2003). As a result, it may strongly interact with anionic molecules within the GIT, such as ingested food components or secreted gastrointestinal components (Y. Chang, McLandsborough, & McClements, 2011). In particular, polylysine may strongly bind to anionic bile salts, phospholipids, or free fatty acids in the GIT, thereby altering lipid digestion and bioavailability.

Recent studies have shown that cationic ε-PL can form strong electrostatic complexes with anionic pectin, which alters its electrical properties, solubility, and aggregation stability (Y. Chang, McLandsborough, & McClements, 2014b; Y. H. Chang, McLandsborough, & McClements, 2012; Lopez-Pena & McClements, 2014). The complexation of oppositely charged biopolymers in foods or the GIT may therefore impact their ability to interact with bile acids.

In this study, isothermal titration calorimetry (ITC) was used to study the interactions between a model bile salt (sodium taurocholate) and ε-PL, pectin and a mixture of these two biopolymers. ITC measures the heat absorbed or released when one solution is titrated into another solution (Doyle, 1997; Freire, Mayorga, & Straume, 1990; Leavitt & Freire, 2001). Previous studies have shown that ITC is an extremely valuable tool for studying the interactions of bile salts with other molecules because it provides valuable data concerning binding enthalpies, critical aggregation concentrations, and binding stoichiometry (Arroyo-Maya & McClements, 2016; Chang et al., 2011; Wangsakan, Chinachoti, & McClements, 2004). In addition, we used electrophoresis, turbidity, and microstructural observations to provide additional information about the nature of the interactions between the bile salts and the two biopolymers.

2. Materials and methods

2.1. Materials

Analytical grade sodium phosphate, hydrochloric acid and sodium hydroxide were purchased from Sigma Chemical Company (St. Louis, MO). Sodium taurocholate was purchased from Fluka Biochemika (Milwaukee, WI). ε-Polylysine (50:50 mixture with dextrin) was provided by Puraq (lot 112022, Puraq Xtend FX50P; Lincolnshire, IL). High methoxyl (DE 69–77%), pectin (lot 512401, TIC Pretested Pectin 1400). All other chemicals were purchased from Sigma-Aldrich Chemical Co. Double distilled water was used to prepare buffer solutions.

2.2. Interaction of bile salt with biopolymers

2.2.1. Solutions preparations

Stock solutions of 2.5% (w/v) bile salt, 0.25, 0.20, 0.125, 0.10, 0.08, 0.05 and 0.02% (w/v) ε-PL, and 0.1 and 0.5% (w/v) pectin were individually prepared by dispersing them in 5 mM phosphate buffer solution (pH 7.0) at room temperature for 12 h to ensure their dispersion and dissolution. After this step, the pH of the stock solutions was readjusted to the required value using diluted solutions of either HCl or NaOH. A pH value of 7.0 was used to simulate the neutral conditions found during intestinal fate.

2.2.2. Isothermal titration calorimetry measurements

To establish the enthalpy change (ΔH) resulting from the interaction of the bile salt with a specific biopolymer, 2.5% (w/v) bile salt solution was titrated into either buffer solution (blank), ε-PL, pectin, or ε-PL-pectin mixed solutions at 37 °C. Twenty-nine 10 μL aliquots of bile salt solution (2.5 w/v %, pH 7.0) were injected sequentially into a 1480 μL titration cell (MicroCalorimeter VP-ITCMicroCal, LLC. Northampton, MA). Each injection lasted 12 s, and there was an interval of 240 s between successive injections. The solution in the titration cell was stirred as a constant speed of 315 rpm throughout the experiments. The resulting heat flow *versus* time curves were integrated using the ITC instrument's software to calculate the interaction enthalpy versus bile salt concentration profiles. Triplicate measurements of the enthalpy profiles were carried out on each sample. The ζ-potential, optical turbidity, and microstructure (observed by optical microscopy) of the solutions were also characterized throughout the titration process.

2.3. Solution characterization

Microelectrophoresis and optical turbidity measurements were used to provide information about the electrical charge and aggregation state