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Real-time measurements to characterize dynamics of emulsion interface during simulated intestinal digestion

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ABSTRACT

Efficient delivery of bioactives remains a critical challenge due to their limited bioavailability and solubility. While many encapsulation systems are designed to modulate the digestion and release of bioactives within the human gastrointestinal tract, there is limited understanding of how engineered structures influence the delivery of bioactives. The objective of this study was to develop a real-time quantitative method to measure structural changes in emulsion interface during simulated intestinal digestion and to correlate these changes with the release of free fatty acids (FFAs). Fluorescence resonant energy transfer (FRET) was used for rapid in-situ measurement of the structural changes in emulsion interface during simulated intestinal digestion. By using FRET, changes in the intermolecular spacing between the two different fluorescent probes labeled emulsifier were characterized. Changes in FRET measurements were compared with the release of FFAs. The results showed that bile salts and pancreatic lipase interacted immediately with the emulsion droplets and disrupted the emulsion interface as evidenced by reduction in FRET efficacy compared to the control. Similarly, a significant amount of FFAs was released during digestion. Moreover, addition of a second layer of polymers at emulsion interface decreased the extent of interface disruption by bile salts and pancreatic lipase and impacted the amount or rate of FFA release during digestion. These results were consistent with the lower donor/acceptor ratio of the labeled probes from the FRET result. Overall, this study provides a novel approach to analyze the dynamics of emulsion interface during digestion and their relationship with the release of FFAs.

1. Introduction

Oil-in-water emulsions have been proposed for encapsulation and delivery of hydrophobic bioactives in food, pharmaceutical and cosmetic industries [1]. In emulsions, the hydrophobic bioactive compounds are often dispersed in the oil phase of emulsions. Release of the encapsulated compounds from these emulsions can be significantly influenced by lipid digestion [2,3]. Lipid digestion occurs primarily in the small intestine of adults [4], where pancreatic lipase acts in concert with various co-factors (co-lipase, bile salts and calcium) to hydrolyze lipids and to produce free fatty acids and monoglycerides [5]. Lipid hydrolysis in emulsions is a dynamic and interfacial process [6], and it is controlled by the interactions of pancreatic lipase, bile salts and its co-factors with the emulsion interface [4,5].

With this motivation, studies have been conducted to modulate the rate of lipid digestion in emulsion formulations using a combination of physical and chemical approaches to engineer the emulsion interface [7–12]. These approaches include (a) layer-by-layer coatings of polymers at the emulsion interface [7,8,10]; (b) increasing steric hindrance using inert polymers at the interface [11] to reduce the rate of binding of bile salts and enzymes such as lipase; and (c) biochemical approaches using modified coating at the emulsion interface to inhibit enzyme activity [12]. Despite significant efforts in designing emulsion interface for controlling the rate of lipid digestion, changes in the emulsion interface upon interactions with bile salts and digestive enzymes are not well understood. Most of our current understanding of the interactions of digestive environment with emulsion interface is based on in-vitro digestion studies. In these in-vitro studies, lipid digestion is usually characterized by measuring the amount of free fatty acids produced based on titration using an alkali solution [13]. To characterize the release of encapsulated compounds from emulsions, the most common approach currently used in these studies is based on centrifugal separation of the micellar phase from the lipid phase

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[13,14]. However, this method often requires high speed centrifugation of the digested emulsion samples [13,15] for an extended period of time that can influence the structure of colloidal particles and the fate of encapsulated compounds. Despite significant efforts in engineering the interface of emulsions and other colloidal particles, the prior studies provide only limited information regarding the dynamics of the interface during digestion.

Interfacial tension measurements are the leading approach commonly used for characterizing dynamics of the oil-water interface during digestion processes [16]. Typically in these measurements, interactions between the suspended oil droplets and the bulk aqueous phase with simulated digestive environments and interfacial active molecules are measured. This approach is useful for understanding the interactions of simulated digestive environment with simple interfacial compositions, but currently this approach cannot be easily translated to measure complex interfacial compositions such as layer-by-layer coatings. In addition, the interfacial tension measurement approaches cannot characterize the role of size of colloidal particles in influencing lipid digestion and release of encapsulated bioactives [5]. Therefore, there are significant gaps in our current knowledge regarding structural changes in the interface of complex emulsion formulations including layer-by-layer coatings during digestion and their relationship with lipid hydrolysis. In order to understand the dynamics of the interface during digestion, there is a need to develop real-time quantitative methods for rapid in-situ analysis and their application to understand dynamics of complex interfacial compositions. It is expected that these measurements provide fundamental understanding of interactions between the interfacial compositions and digestion processes including enzymatic processes and biochemical interactions. Furthermore, these measurements may not only bridge the gap between the interfacial tension measurements and the current design of in-vitro digestion studies, but also address some of the limitations of both the approaches.

The focus of this study was to understand structural changes in emulsion interface during simulated intestinal digestion and to correlate these structural changes with the release of free fatty acids from emulsion formulations. For characterizing changes in emulsion interface during digestion, an optical method based on fluorescence resonance energy transfer (FRET) was developed. FRET is a distance dependent non-radiative energy transfer process between the donor and the acceptor fluorophores [17,18]. This energy transfer from the donor to the acceptor fluorophore is mediated by intermolecular long-range dipole–dipole coupling between the fluorophores [17,18]. FRET is highly efficient if the donor and acceptor fluorophores are positioned within the Förster radius (the distance at which half the excitation energy of the donor is transferred to the acceptor, typically 3–6 nm) [18] and with appropriate alignment of the dipoles. To monitor changes in emulsion interface using FRET, phospholipid emulsifier molecules conjugated with two different fluorescent probes were selected. Changes in the relative positioning of these probes can be tracked in real-time by measuring the ratio of fluorescence intensity of the selected donor and acceptor probes using the donor excitation wavelength. The food-grade lecithin and bile salts stabilized emulsion labeled with FRET pair fluorophores at the interface was selected as a model system to evaluate the dynamics of phospholipid interface during simulated intestinal digestion. To evaluate the influence of a biopolymer coating on the dynamics of an emulsion interface during digestion, the model emulsion was coated with food grade polymers ε-polylysine (MW around 5000 Da) and medium molecular weight chitosan (MW around 190–310 kDa) respectively based on electrostatic interactions. ε-Polylysine has been proposed to inhibit lipase activity and thus may influence the rate of lipid digestion [12,19]. Chitosan is an indigestible polysaccharides [20] and prior studies have indicated that chitosan could interfere with lipid digestion [10]. This is due to the gel forming ability or enhanced viscosity of chitosan coatings as has been suggested in some of the prior studies [21,22]. Dynamics of the emulsion interface measured based on FRET measurements during simulated intestinal digestion was correlated with the release of fatty acids from the model emulsions.

In summary, this study (a) develops a real-time quantitative method to measure structural changes in emulsion interface during simulated intestinal digestion and correlate these changes with the release of free fatty acids; and (b) evaluates the role of biopolymer membrane coatings on emulsion interface in influencing interactions between the interface and simulated intestinal fluid and its individual components. The unique approaches developed in this study will enable detailed understanding of real-time dynamics of emulsion interface during simulated intestinal digestion. These measurements will enable rational design of encapsulation systems to control the release of encapsulated compounds.

2. Materials and methods

2.1. Materials

Bile salts (~50% cholic acid sodium salt and ~50% deoxycholic acid sodium salt), calcium chloride, low molecular weight chitosan (MW around 190–310 kDa), and lipase from porcine pancreas (type II) were obtained from Sigma–Aldrich (St. Louis, MO, USA). ε-Polylysine (EPL, MW around 5000 Da) was a gift from Purac America (Lincolnshire, IL, USA). Oregon Green® 488 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (OG-ph) and Texas Red® 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine, triethylammonium salt (TR-ph) were purchased from Life Technologies (Carlsbad, CA, USA). Low melting lecithin (ALCOLEC®PC 75) was a gift from American Lecithin Inc. (Oxford, CT, USA). Organic canola oil was a gift from Spectrum Naturals, Inc. (Petaluma, CA, USA). Sodium chloride, tris hydrochloride (pH 7.5) with high purity low metal, sodium hydroxide and chloroform were obtained from Fisher Scientific. Ultra-pure water (16 MΩ-cm) was obtained from an in-house water filtration system.

2.2. Emulsion preparation

2.2.1. Lecithin–bile primary emulsion

Oregon Green 488 labeled 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (OG-ph) and Texas Red labeled 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine, triethylammonium salt (TR-ph) were selected as the donor and the acceptor of FRET pair, respectively. Lecithin and bile salts were dissolved in water and the final concentrations of lecithin and bile salts were 1.5 w/v % and 0.5 w/v %, respectively. Bile salts were selected to stabilize the lecithin emulsion based on their net negative charge. The concentration of bile salts used was below the CMC (critical micelle concentration) of bile salts, thus cannot disrupt the emulsions. A stock OG-ph or TR-ph solution was prepared by dissolving OG-ph or TR-ph in chloroform. The concentration of the dye labeled phospholipids was 0.1 mol% compared to the total amount of lecithin used. The dye labeled phospholipid solution was dried in a glass vial under a stream of nitrogen then placed in a vacuum desiccator for 1 h. The lecithin and bile salts solution was mixed with the OG-ph or TR-ph after drying. Coarse emulsions were prepared by dispersing canola oil (4 w/v %) in lecithin and bile salt solution using a hand-held disperser (Ultra-Turrax model T25, IKA Works, Wilmington, NC, USA) set at 9500 rpm for 2 min. The coarse emulsion was then probe-tip sonicated for 30 s at 50% of maximum amplitude (55 W power) with a Qsonica Q55 ultrasonic processor (Newton, CT, USA).