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Control of the gastrointestinal digestion of solid lipid nanoparticles using PEGylated emulsifiers

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ABSTRACT

We prepared solid lipid nanoparticles (SLNs) with tristearin and various emulsifiers which had different chain length PEGs (10–100 times-repetition of ethylene glycol) to control their digestion fate in the gastrointestinal tract. Fabricated SLNs after acidic/high-ionic-strength media treatment were stable regardless of the ζ-potential (ZP) disappearance. Additionally, highly PEGylated SLNs successfully hindered the adsorption of both bile acid (BA) and lipase on the SLN surface, while lowly PEGylated SLNs interrupted that of only lipase. In simulated small intestinal fluid, lipolysis of highly PEGylated SLNs increased with decrease of the emulsifier density on the SLNs, whereas lipolysis of lowly PEGylated SLNs increased with decrease of the particle size. These results suggested that high PEGylation was more efficient than low PEGylation to hinder the lipolysis initiated from the competitive replacement of the SLN-covering emulsifiers with BAs. Consequently, the SLN digestion could be controlled by choosing the length and concentration of PEGylated emulsifiers.

1. Introduction

Controllable digestion of a lipid carrier system in the gastrointestinal tract (GIT) is a large issue that needs to be solved to successfully develop functional foods that are fortified to increase biological activities, as digestion effects the bioavailability and controlled release of bioactive materials incorporated into carriers (McClements, Decker, & Park, 2008; Porter, Pouton, Cuine, & Charman, 2008). Ingested lipid carrier systems should travel from the mouth to intestine along the lumen and experience various environmental changes, e.g., mouth: neutral pH, high ionic strength, mucin, amylase, lingual lipase and so on; stomach: low pH, high ionic strength, mucin, pepsin, gastric lipase and so on; and small intestine: neutral pH, high ionic strength, pancreatic lipase, colipase, bile salts and phospholipids (Kong & Singh, 2008; McClements & Xiao, 2012). During the retention time in the GIT, the acidic conditions in the stomach could bring the unwanted aggregation of the carriers, and lingual, gastric, or pancreatic lipase could hydrolyze the lipid molecules in the carriers. Particularly, lipid digestion occurs mainly in the small intestine (70–90%) by pancreatic lipases with the help of calcium ions, bile salts and colipase (Maldonado-Valderrama, Wilde, Macierzanka, & Mackie, 2011). Therefore, the controllable digestion of the lipid carriers can be accomplished by preventing the collision among the lipid droplets and the action of various lipases, colipase, and bile salts at the lipid-water interface. In this regard, understanding this interface of lipid carrier systems would be a key point to modulate their GIT digestion.

Many researchers have strived to control the lipid hydrolysis by means of modulating the interfacial properties. Maldonado-Valderrama et al. examined the compositional changes of the β-lactoglobulin-stabilized lipid surface by ionic surfactants (Tween 20) and biological surfactants (bile salts) during the digestion process (Maldonado-Valderrama, Gunning, Ridout, Wilde, & Morris, 2009; Maldonado-Valderrama, Gunning, Wilde, & Morris, 2010; Maldonado-Valderrama et al., 2008). Chu et al. studied the interfacial changes induced by bile salts and the adsorption of colipase and pancreatic lipase onto the interface, and then suggested that galactolipids on the lipid surface could slow the rate and extent of lipid digestion in the GIT (Chu et al., 2009, 2010). Furthermore, the controllable digestion method of oil droplets under *in vitro* small intestinal conditions was introduced using a non-ionic emulsifier (Poloxamer 188) (Torcello-Gómez, Maldonado-Valderrama,

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Martín-Rodríguez, & McClements, 2011). These literature examples are representative instances to demonstrate the possibility of controllable lipid digestion by modulating the design of the interfacial composition. However, it is still unclear what size and quantity of emulsifiers at the interface can effectively hinder the action of active components on the biological surface, such as bile salts, colipase and lipase.

The solid lipid nanoparticle (SLN) system has been regarded as an attractive lipid carrier system due to the use of solid-state lipid at room/body temperatures. The solid lipid in SLNs has some merits for delivering bioactive materials, i.e., a rigid matrix capable of protection from the outside and offering the possibility for the controlled release of the bioactive components (Müller, Mäder, & Gohla, 2000). Polyethylene glycol (PEG) is a synthetic polymer approved for its safety in the body (Nagaoka & Nakao, 1990), which has been universally used in pharmaceutics and cosmetics, as well as in foods. PEGs with large chains above a certain level (~2 kDa) can sterically repel the approach of proteins, including enzymes, and have the ability to avoid detection by the immune system in the body (Jeong, Park, & Kim, 2011). In this manner, many molecules or macrostructures are covalently/non-covalently attached with PEGs to have a "stealth function" in the body, which is called as PEGylation (Gref et al., 2000; Niidome et al., 2006). Müller and coworkers achieved the modulation of the lipolysis of PEGylated SLNs under simulated GIT conditions using Poloxamer 188 (Müller, Rühl, & Runge, 1996) and Poloxamer 407/cholic acid (Olbrich & Müller, 1999). However, the colipase/lipase adsorption and surfactant displacement on the lipid surface were unclear. Therefore, the mechanisms of SLN digestion should be verified, along with considering the compositional changes of the interface at a molecular level.

In this study, SLNs prepared using tristearin (TS) and various PEGylated emulsifiers were utilized, and the number of emulsifiers participating in covering the SLN surface was quantitatively determined for consideration on a molecular level. Moreover, under the mimicked *in vitro* GIT environment, the digestion patterns of the PEGylated SLNs were monitored to determine the effects of the type/concentration of PEGylated emulsifier at the lipid-water interface on the SLN digestion, followed by the action of bile acids (BAs), colipase and pancreatic lipase. On the basis of the obtained results, we suggested the digestion mechanism of PEGylated SLNs on a molecular level, as well as the controllable hydrolysis methods of the SLNs.

2. Materials and methods

2.1. Chemicals

Glyceryl tristearate (tristearin), polyoxyethylene (10) stearyl ether (PEG10SE, Brij® S10), polyoxyethylene (100) stearyl ether (PEG100SE, Brij® S100), polyoxyethylene (10) oleyl ether (PEG10OE, Brij® O10), decaethylene glycol monododecyl ether (PEG10LE) and polyoxyethylene sorbitan monostearate (PEG20SS, Tween® 60) were purchased from the Sigma Aldrich Co. (St. Louis, MO, USA). Polyethylene (10) stearate (PEG10S) and polyoxyethylene (100) stearate (PEG100S, Myrj® S100) were obtained from TCI (Tokyo, Japan) and Croda (Parsippany, NJ, USA), respectively. All other chemicals were of analytical reagent grade.

2.2. Solid lipid nanoparticle preparation

The SLNs were prepared using an oil-in-water emulsion technique with a high-speed blender and sonication probe as suggested previously by our group (Ban, Lim, Chang, & Choi, 2014), with slight modifications. First, the lipid (5 wt%) and aqueous (95 wt%) phases were heated to 95 °C and mixed using a high-speed blender (Ultra-Turrax T25D, Ika Werke GmbH & Co., Staufen, Germany) at 8,000 rpm for 1 min and then at 11,000 rpm for 1 min while being maintained at 95 °C. The lipid phase of the SLNs was composed of TS, while the aqueous phase was fabricated by adding PEGylated emulsifiers until reaching the pre-determined concentration (5.331, 17.058, 25.588, 34.117, or 46.910 mM of the entire SLN system) in double-distilled water (DDW) containing 0.02 wt% sodium azide with mixing for 1 h. After preparing the coarse oil-in-water emulsion, the droplet size was further reduced by sonication (VCX 750, Sonics & Materials Inc., Newtown, CT, USA) for 4 min at 60% amplitude, a duty cycle of 1 s, and 95 °C. After reducing the droplet size, post-sonication was applied for 6 min to the emulsions during cooling to 25 °C in a jacketed beaker, and the samples were maintained at room temperature (25 °C).

2.3. Quantifying non-aggregated solid lipid nanoparticles (yield %)

SLNs diluted 10-fold with DDW were passed through a 1 μm pore size glass microfiber filter (GF/B, Whatman Ltd., Loughborough, UK). The aggregated SLNs remaining on the filter (micron-scale) were weighed after drying in an oven at 50 °C. The difference in filter weight before and after the procedure, which was the weight of the creamed or aggregated SLNs, was recorded.

2.4. Measuring the solid lipid nanoparticle size and ζ-potential

The prepared SLNs (4.5 ml) were diluted with 40.5 ml of DDW in a vial to separate the layers containing the aggregated and non-aggregated SLNs. The vial containing the diluted samples was sealed tightly with a screw cap and incubated overnight at ambient temperature. The aggregated SLNs were removed by filtration with a 1 μm pore size glass microfiber filter (GF/B, Whatman Ltd.), and the mean size (z average) of the passed particles was measured using a Zetasizer (Nano ZS, Malvern Instruments Ltd., Worcestershire, UK) operated at a 173° angle with a helium-neon laser (λ = 633 nm). In addition, the ζ-potential (ZP) was also measured using the Zetasizer. The ZP measurement was based on the Smoluchowski equation at 25 °C with an electric field strength of 20 V cm^{-1}.

2.5. Determining the emulsifier surface load

Using an assumption of a spherical shape for all of the SLNs (Fig. S1 in Supplementary Material), the emulsifier surface load (Γ_s) was calculated as follows: $\Gamma_s = C_a D/6\Phi_v$, where C_a is the concentration of the emulsifier adsorbed onto the surfaces of SLNs, D is the mean diameter (z average), and Φ_v is the lipid phase volume fraction (i.e., 0.05 lipid phase) (McClements, 2007). C_a was measured by subtracting the concentration of emulsifiers suspended as single molecules or micelles from the initial concentration of total emulsifiers in the aqueous phase. A total of 2.5 ml of the previously diluted and filtered SLN dispersion system was injected into the Sephadex G-25 column (GE Healthcare, Chalfont St Giles, UK) filled with DDW. Next, 1 ml of DDW was serially added, and then each fraction passing through the column was collected in a micro-tube. Subsequently, aliquots of the fractions in the fifth and sixth tubes were selected as samples in which the emulsifier molecules did not participate in any emulsifying activity. Colorimetry for the quantification of PEGylated emulsifiers has been reported previously (Khossravi, Kao, Mrsny, & Sweeney, 2002). Briefly, each sample was dried at 60 °C in an oven. The sample was then cooled in ambient conditions, and 0.6 ml of ammonium cobaltothiocyanate (ACTC) solution as well as 1.2 ml of dichloromethane were added. The ACTC solution was prepared using 3 g of cobalt nitrate hexahydrate and 18 g of ammonium thiocyanate in 100 ml of DDW. Samples were vortexed for 10 s