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Disposition and crystallization of saturated fatty acid in mixed micelles of relevance to lipid digestion

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ABSTRACT

During lipolysis of triglyceride by lipase, monoglyceride and fatty acids are produced which combine with gastrointestinal fluids to form self-assembled structures. These solubilize hydrophobic food components to promote their absorption. The aim of this study was a detailed understanding of structure formation from triglyceride digestion products with saturated short-, medium- and long chain fatty acids. Complementary characterization methods have been applied comprising small angle X-ray and neutron scattering – the latter involving the contrast matching technique using fully deuterated fatty acids – polarized and depolarized dynamic light scattering and cryogenic-transmission electron microscopy. Shape, size and solubilization capacity of the self-assembled structures was dependent on composition and lipid chain length. Crystallization of fatty acid was observed when the solubility limit in the mixed bile salt micelles was exceeded; however, increasing pH and temperature increased the fatty acid solubility. The results provide insight into structure formation and crystallization of incorporated lipolysis products; this is important for a detailed understanding of food structure and nutrition, as well as the rational design of lipid based drug delivery systems.

1. Introduction

Triglyceride lipids are responsible for 30% of the daily calorie intake in the Western diet [1,2]. The digestion of triglycerides by lipases in the gastro-intestinal tract leads to generation of fatty acids and monoglycerides. These digestion products are required for membrane synthesis, elements of cells and tissues, production of signaling compounds and a source of energy [1]. The disposition of fatty acids may impact on lipid metabolism with implications for metabolic diseases such as atherosclerosis, type 2 diabetes and obesity [3]. Fatty acids can also affect the development and progression of cancer and cardiovascular disease [4–6].

In the small intestine, fatty acid and monoglyceride combine with endogenous amphiphilic molecules secreted from the gall-bladder, such as bile salt, cholesterol and phospholipid. These mixtures spontaneously self-assemble into thermodynamically stable structures such as mixed micelles of varying size and shape, vesicles and more complex liquid crystalline phases [7–9]. This structure formation has been confirmed *in vivo* in killifish [10] and human duodenal aspirates following a lipid-rich meal [9,11–13]. The phase behavior has been studied in systems containing combinations of bile salt, monoglyceride and cholesterol, where it has been shown that colloidal structure and size depends on composition [14–17]. Bile salt micelles have been shown to elongate with increasing proportions of phospholipid, finally resulting in vesicles [18–20]. Bile salt micelles have also been found to increase in size with addition of monoglyceride and fatty acid [7].

Abbreviations: Cryo-TEM, cryogenic-transmission electron microscopy; DLS, dynamic light scattering; DDLS, depolarized dynamic light scattering; SANS, small angle neutron scattering; SAXS, small angle X-ray scattering; BS, bile salt; MG, monoglyceride; FA, fatty acid.

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Such structures facilitate transport of hydrophobic molecules in the gastrointestinal tract and uptake through the enterocyte membrane. They have high solubilizing capacity for hydrophobic molecules including cholesterol, dietary lipids [21,22] and fat-soluble vitamins A, D, E and K [23,24], and thus act as their carrier to prevent precipitation and enhance bioavailability. They also have a high solubilization capacity for hydrophobic drugs, which makes them potential vehicles for drug delivery [7,25]. Co-administration of drugs with lipids has been proposed to reduce precipitation of the former during lipid digestion and increase bioavailability, rendering them of interest in the drug delivery field [26,27].

Many saturated triglycerides are present in the Western diet [28], and the fatty acids produced on their digestion in the gut have melting points higher than physiological temperature e.g. C_{12}, C_{14}, C_{16} and C_{18} [29]. They are protonated at physiological pH, which can result in crystallization of lipid and excretion in the faeces, rather than absorption [30,31]. The melting points of C_8 and C_{10} fatty acids are lower than physiological temperature enabling them to be better solubilized and absorbed than long chain fatty acids. However there is a lack of understanding of the influence of structure formation on crystallization of saturated fatty acids and monoglycerides in biologically relevant multi-component systems containing bile salt, phospholipid, monoglyceride and fatty acid under physiological pH conditions. Solubilization of lipid digestion products such as fatty acids is required for uptake and use by the body; thus crystallization is undesirable. In this study, the shape and size of the resulting colloidal structures and the crystallization of saturated fatty acids in biologically relevant mixtures have been studied using scattering methods and electron microscopy.

The pH in the gastrointestinal tract has been shown to vary between 2 and 8 [32] (Fig. 1), which will influence its partition coefficient between the aqueous bulk phase and the lipid areas of the colloidal structure, and potentially induce crystallization of the fatty acid. In addition, pH has an impact on the protonation state of the fatty acid which can modify the packing geometry based on the effective charge per molecule at the interface [33–36], thus influencing the size and shape of structures.

To the best of our knowledge, this is the first study investigating the disposition and crystallization of lipolysis products in mixed micellar structures in biologically relevant conditions of the human intestine. An understanding of how intermolecular assembly of digestion products is influenced by composition, lipid chain length, pH and temperature will provide insight into the digestion process, and further the understanding for the delivery of hydrophobic molecules, including drugs, to the body.

2. Experimental methods

2.1. Materials

Deuterated caprylic acid (C_8 fatty acid, $C_8HD_{15}O_2$), lauric acid (C_{12} fatty acid, $C_{12}HD_{23}O_2$), and myristic acid (C_{14} fatty acid $C_{14}HD_{27}O_2$), were prepared as described below. Monomyristin (C_{14} monoglyceride > 97%) was obtained from TCI Co., Ltd. (Kawaguchi, Saitama, Japan). Bile salt (sodium taurodeoxycholate > 95%), monocaprylin (C_8 monoglyceride 99%), monolaurin (C_{12} monoglyceride 99%), tris maleate (reagent grade), NaOH (p.a. grade) and HCl (p.a. grade) were purchased from Sigma Aldrich (St. Louis, MO, USA). Phospholipid (1,2-dioleoyl-sn-glycero-3-phosphocholine, DOPC) was from Trapeze Association Pty. Ltd., (Clayton, Victoria, Australia). Sodium chloride was purchased from Chem Supply (Gillman, SA, Australia). Sodium azide was purchased from Merck Schuchardt OHG (Eduard-Buchner-Straße, Hohenbrunn, Germany). Ultra-pure water (resistivity > 18 MΩ cm) was used for the preparation of all samples.

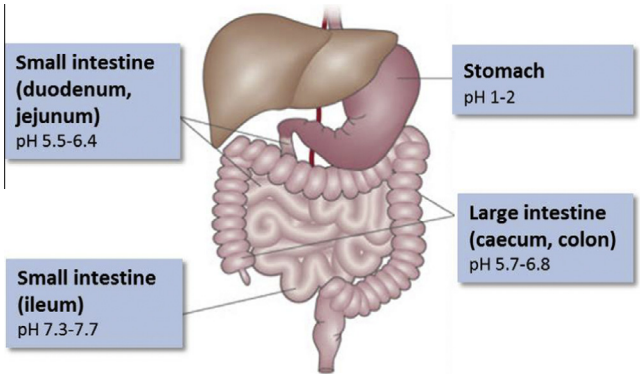

Fig. 1. Variation in pH in the gastrointestinal tract. Image adapted with permission from [37].

2.2. Deuteration method

The deuteration of the three fatty acids were achieved following a procedure published elsewhere [36]. In summary, a mixture of the appropriate acid, Pt/activated carbon and NaOD in D_2O was subjected to two hydrothermal H/D exchange cycles in a Parr pressure reactor at 220 °C (23 bar) for 3 days each. Thin layer chromatography was used (referenced with the protonated compound) to develop separation protocols. Purification of the deuterated compounds was performed on silica gel columns, eluted with the appropriate solvents. ^{1}H NMR (400 MHz), and ^{2}H NMR (61.4 MHz) spectra were recorded on a Bruker 400 MHz spectrometer at 298 K. More details on the characterization are presented in the Supporting information. The ^{1}H NMR and ^{2}H NMR spectra, as well as the mass spectrometry data are shown in Figs. SI-1–SI-6.

2.3. Sample preparation

Fed simulated intestinal fluid was prepared using bile salt (BS, sodium taurodeoxycholate) and phospholipid (PL, DOPC) concentrations at a ratio of 20 mM:5 mM to represent concentrations relevant after food intake, in digestion buffer (50 mM Tris maleate, 150 mM NaCl, 6 mM NaN_3 as an antibacterial agent) [38–41]. The PL was weighed into a round bottom flask and dissolved in chloroform which was subsequently evaporated to produce a thin film of PL. BS and digestion buffer was added, and the PL and BS were dispersed in a sonicator bath for 30 min. The equilibrium systems reflecting the endpoint of the lipid digestion process were prepared by adding 10 mM monoglycerides (C_8, C_{12} or C_{14}) and 20 mM deuterated fatty acids (C_8, C_{12} or C_{14}) [7,42–44] to high levels of bile salt/phospholipid (BS/PL) micelles expected in the fed state simulating the gastrointestinal state at the nominal end point of digestion of triglycerides (C_8, C_{12} or C_{14}). The 1:2 ratio of monoglyceride to fatty acid represents the nominal end point of digestion of the corresponding triglyceride, and thus is most commonly used. However there is evidence that digestion can proceed past this, thus it may not necessarily be entirely reflective of the end point of digestion but some transient composition towards the end of digestion [9,43,45,46].

Sonication with a tip sonicator for 30 s at 100 W was used to solubilize the components. Samples were equilibrated for 24 h and pH was adjusted with concentrated NaOH and HCl.

2.4. Small angle neutron scattering (SANS)

SANS experiments were performed on the Quokka instrument at OPAL [47] at a wavelength of 5.0 Å and 10% wavelength resolution. Two instrument configurations were used with a