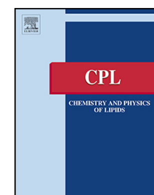
Chemistry and Physics of Lipids 190 (2015) 43–50

Contents lists available at ScienceDirect

Chemistry and Physics of Lipids

journal homepage: www.elsevier.com/locate/chemphyslip



Selective deuteration for molecular insights into the digestion of medium chain triglycerides

Stefan Salentinig[a,**], Nageshwar Rao Yepuri[b], Adrian Hawley[c], Ben J. Boyd[a], Elliot Gilbert[d], Tamim A. Darwish[b,*]

[a] Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash University (Parkville Campus), 381 Royal Parade, Parkville, VIC 3052, Australia
[b] National Deuteration Facility, Australian Nuclear Science and Technology Organisation, Locked Bag 2001, Kirrawee DC, NSW 2232, Australia
[c] SAXS/WAXS Beamline, Australian Synchrotron, 800 Blackburn Rd, Clayton, VIC 3168, Australia
[d] Bragg Institute, Australian Nuclear Science and Technology Organisation, Locked Bag 2001, Kirrawee DC, NSW 2232, Australia

ARTICLE INFO

Article history:
Received 26 May 2015
Received in revised form 29 June 2015
Accepted 30 June 2015
Available online 4 July 2015

Keywords:
Selective deuteration
Medium chain triglycerides
Pancreatic lipase
Enzymatic interesterification
Tricaprin
Monocaprin
Capric acid
Deuterated lipids

ABSTRACT

Medium chain triglycerides (MCTs) are a unique form of dietary fat that have a wide range of health benefits. They are molecules with a glycerol backbone esterified with medium chain (6–12 carbon atoms) fatty acids on the two outer (*sn-1* and *sn-3*) and the middle (*sn-2*) positions. During lipid digestion in the gastrointestinal tract, pancreatic lipase stereoselectively hydrolyses the ester bonds of these triglycerides on the *sn-1* and *sn-3* positions resulting in *sn-2* monoglyceride and fatty acids as major products. However, the *sn-2* monoglycerides are thermodynamically less stable than their *sn-1/3* counterparts. Isomerization or fatty acid migration from the *sn-2* monoglyceride to *sn-1/3* monoglyceride may occur spontaneously and would lead to glycerol and fatty acid as final products. Here, tricaprin (C10) with selectively deuterated fatty acid chains was used for the first time to monitor chain migration and the stereoselectivity of the pancreatic lipase-catalyzed hydrolysis of ester bonds. The intermediate and final digestion products were studied using NMR and mass spectrometry under biologically relevant conditions. The hydrolysis of the *sn-2* monocaprin to glycerol and capric acid did not occur within biologically relevant timescales and fatty acid migration occurs only in limited amounts as a result of the presence of undigested diglyceride species over long periods of time in the digestion medium. The slow kinetics for the exchange of the *sn-2* fatty acid chain and the stereoselectivity of pancreatic lipase on MCTs is relevant for industrial processes that involve enzymatic interesterification and the production of high-value products such as specific structured triacylglycerols, confectionery fats and nutritional products.

© 2015 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Dietary triglycerides are water-insoluble lipids, which are converted to more polar products during digestion for absorption. The glycerol backbone of the triglycerides allows three stereochemically distinct fatty acids positions: the two outer (*sn-1*,*sn-3*) and the middle position (*sn-2*). Digestion catalyzed pancreatic lipase hydrolyzes triglycerides stereospecifically to *sn-2* monoglyceride and free fatty acids (Borgström, 1964). These components are physiologically-relevant as only the fatty acid at the *sn-2* position can be absorbed intact as *sn-2* monoglyceride whereas the fatty acids at *sn-1,3* positions in the triglycerides are absorbed as free fatty acids that metabolize independently. Fatty acids and monoglycerides enter the enterocytes as monomers. There they are resynthesized to triglycerides by the action of the triglyceride synthetase complex (Voet and Voet, 1995). Some *sn-2* monoglyceride might ultimately serve as the backbone for gut or liver phospholipids, exerting downstream influence on lipid metabolism (Hayes, 2001). However, the *sn-2* monoglycerides are thermodynamically less stable than their *sn-1/3*counterparts. Isomerization or fatty acid migration from the *sn-2* monoglyceride to *sn-1* or *sn-3* monoglyceride may occur spontaneously and would lead to glycerol and fatty acid as final products. More than 30% acyl migration was found in *sn-1,2* diglycerides after 24 h at 37 °C (Kodali et al., 1990). The resulting equilibrium distribution in long chain monoglycerides (fatty acid chain length >14) comprises of 9% *sn-2* and 91% *sn-1*, with shorter chain fatty acids migrating faster

* Corresponding author.
** Corresponding author.
E-mail addresses: stefan.salentinig@gmail.com (S. Salentinig), tamim.darwish@ansto.gov.au (T.A. Darwish).

http://dx.doi.org/10.1016/j.chemphyslip.2015.06.007
0009-3084/© 2015 Elsevier Ireland Ltd. All rights reserved.

than longer chain fatty acids (Laszlo et al., 2008; Compton et al., 2007; Boswinkel et al., 1996).

The formation of *sn-2* monoglyceride in equilibrium conditions is enhanced up to 80% yield by the presence of self-assembled structures (Kodali et al., 1990; Holmberg and Osterberg, 1988; Holmberg and Österberg, 1990; Mazur et al., 1993). This is biologically-relevant, as these structures such as mixed micelles and more complex liquid crystalline structures are observed in monoglyceride/free fatty acid/bile salt mixtures (Hernell et al., 1990; Hofmann and Borgström, 1964; Mazer et al., 1980; Schurtenberger et al., 1985; Salentinig et al., 2014) and during the *in-vitro* digestion of triglyceride emulsions (Salentinig et al., 2011) and milk fat (Salentinig et al., 2013; Salentinig et al., 2015). These structures also form the basis for the transport and absorption of lipids and support the solubilisation and absorption of oil-soluble food components (*e.g.*, carotenes, vitamin A, D, E, and K) in the aqueous system of the gastrointestinal tract (Salentinig et al., 2014; Salentinig et al., 2011; Salentinig et al., 2013; Salentinig et al., 2010). Compared to molecular transport, micelles and vesicles enhance the number of fatty acid and monoglyceride molecules available for uptake by the enterocytes. From a monomeric point of view, fatty acid is transferred 3-fold faster than the corresponding monoglyceride under pH conditions of the small intestine (Narayanan and Storch, 1996). The size of the fatty acid is extremely important as the physiological effects of medium-chain fatty acids are distinctly different from the long-chain fatty acids more commonly found in our diet. Medium-chain triglycerides (MCTs) are generally considered a good biologically inert source of energy that humans find relatively easy to metabolize and are a common excipient in pharmaceutical lipid-based formulations (Bach and Babayan, 1982). Until the early 1980s, MCTs were predominantly available only as a constituent of butter, coconut oil, and other natural sources. However, since that date, processes have been developed to produce them in large quantities to be used primarily for therapeutic application in a number of conditions and for the treatment of disorders of lipid absorption. Thus a complete understanding of the MCT digestion process on the molecular level is imperative. Knowledge and understanding of how these food and supplement components are treated and utilized during the digestion process opens opportunities for functional food products that may help to avoid or cure health issues including obesity, vitamin malabsorption, the risk of coronary heart disease and cancer (Austin, 1991; Unger and Zhou, 2001; Katan, 2000). However, there is a lack of information on the stereoselectivity and fatty acid chain dynamics in digestion processes catalysed by pancreatic lipase. This information is also important in industrial processes that utilize lipase technology for the modification of oils and fats to produce high-value added products, such as cocoa butter equivalents, human milk fat substitutes, and other specific-structured lipids. Enzymatic interesterification is a catalytic reaction that occurs when an enzyme is introduced into oil and rearranges the fatty acids on the glycerol backbone of a triglyceride (Xu, 2000). For example, tripalmitin treated with oleic acid in the presence of 1,3-specific pancreatic lipase gives products where the palmitate is retained at the *sn-2* position, whereas oleate is introduced at *sn-1* and *sn-3*, producing a human milk fat substitute such as Betapol. In practice, pure starting materials are not used (Akoh and Xu, 2002).

Here we report the synthesis of selectively deuterated tricaprin, a medium chain length triglyceride, to study the intermediate and final products of the *in situ* digestion by pancreatic lipase using ^{1}H and ^{2}H NMR and mass spectrometry (MS) under biologically relevant conditions (pH, T, bile salt and lipase concentration). The use of selective deuteration provides a direct method to identify unequivocally the final products of digestion of this symmetrical triglyceride using mass spectrometry. A combination of (i) fully-protonated tricaprin; (ii) tricaprin with fully-deuterated fatty acids on the *sn-1/3* positions, a deuterium atom on the *C2* position of the glycerol backbone, and non-deuterated fatty acid on position *sn-2*; and (iii) tricaprin with the fully deuterated fatty acid on the *sn-2* position and non-deuterated fatty acid on the *sn-1/3* were used to investigate the stereoselectivity of the lipase action, fatty acid chain migration and enable product determination with mass spectrometry. In mass spectrometry, deuterated adducts occur at higher *m/z* values compared to their hydrogenated counterparts as a result of the mass difference of deuterium and hydrogen. As a consequence, mass spectra from the hydrolysis of the deuterated components provides detailed *qualitative* information on the stereochemistry of the hydrolysis reaction. In the absence of deuteration, mass spectrometry would not be able to determine the identity of the structural isomers of the digested products due to the similarity in the mass values of the different isomers of monoglycerides or diglycerides. For NMR, deuteration provides another nucleus (*i.e.*, ^{2}H) and frequency dimension, which allows cleaner spectral windows to be probed for changes when a mixture of hydrogen containing reagents is used: the ^{2}H labeled fatty acid chains of interest in the triglycerides can be solely probed without any interference from the ^{1}H signals of the other components. This approach allows direct observation of the signals during the digestion process and allows monitoring of the appearance and disappearance of signals and assists in confirming the identity of the final digestion products that are determined by mass spectrometry.

2. Materials and methods

2.1. Materials

Selectively-deuterated tricaprin with two fully-deuterated capric acid chains on the *sn-1* and *sn-3* positions and one deuterium atom on the *C2* position of the glycerol backbone, as well as deuterated tricaprin with fully-deuterated fatty acid chain on the *sn-2* position were synthesized as described below. Tricaprin ($C_{33}H_{62}O_{6}$, >98%) was purchased from Tokyo Chemical Industry Co., Ltd., Tokyo, Japan. Bile salt (sodium taurodeoxycholate >95%), NaOH, NaOD, HCl and DCl (p.a. grade) were purchased from Sigma Aldrich (St. Louis, MO, USA). Pancreatin extract (USP grade pancreatin activity) was from Southern Biologicals (Nunawading, Victoria, Australia). D_2O (99.8%) was supplied by AECL (Ontario, Canada). Ultra-pure water (resistivity >18 MΩ cm) was used for the preparation of all samples.

The deuteration of the capric acid was performed by hydrothermal reactions using a Mini Benchtop 4560 Parr Reactor (600 mL vessel capacity, 3000 psi maximum pressure, 350 °C maximum temperature). Thin layer chromatography was used (referenced with the protonated compound) to estimate the purity and to develop separation protocols. ^{1}H (400 MHz) and ^{2}H NMR (61.4 MHz) spectra were recorded on a Bruker 400 MHz spectrometer at 298 K. Chemical shifts, in ppm, were referenced to the residual signal of the corresponding NMR solvent. Deuterium NMR was performed using the probe's lock channel for direct observation.

Electrospray ionization mass spectra (ESI–MS) were recorded for the deuterated fatty acid on a 4000 QTrap AB Sciex spectrometer. The overall percentage deuteration of the molecules was calculated by MS using the isotope distribution analysis of the different isotopologues. This was calculated taking into consideration the ^{13}C natural abundance, whose contribution was subtracted from the peak area of each M + 1 signal to allow for accurate estimation of the percentage deuteration of each isotopologue.

Download English Version:

https://daneshyari.com/en/article/1251679

Download Persian Version:

https://daneshyari.com/article/1251679

Daneshyari.com