COMMUNICATIONS

Stereospecificity of Premature Human Infant Lingual Lipase[1]

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ABSTRACT

The lingual lipase in gastric aspirates from premature infants was found to be partially stereospecific for *sn*-3 esters of synthetic enantiometric triacylglycerols containing 18:1 and 16:0. The *sn*-3 ester was hydrolyzed about 4 times faster than the acid at the *sn*-1 position with no difference in rates between 18:1 and 16:0. The *sn*-2 was also hydrolyzed to some extent.
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INTRODUCTION

Lingual lipase is secreted from von Ebner's glands located on the posterior surface of the tongue in humans and other species (1). The enzyme starts digestion of dietary TG in the stomach, producing DG, MG, FFA and glycerol, and contributes significantly to lipolysis of dietary TG in preterm and term infants whose pancreatic function and syntheses of bile salts have not fully developed. The delivery of the MG and FFA formed by lingual lipase into the small intestine should initiate the immediate formation of mixed micelles followed by rapid absorption. The enzyme assists in the digestion of milk fat beyond initial lipolysis. Native milk fat globules have been reported as being resistant to the action of pancreatic lipase under the conditions studied but prior exposure of the globules to lingual lipase increased the rate of lipolysis (2). The enzyme therefore enhances absorption of dietary lipids at a crucial period in the infant's life.

The identity of the digestion products passed from the stomach into the small intestine will be controlled in part by the specificity of lingual lipase. Rat lingual lipase is partially specific for primary esters of TG and for the *sn*-3 as compared to the *sn*-1 ester (3). This latter type of preferential lipolysis has been termed stereospecificity. We have investigated the stereospecificity of lingual lipase from premature infants using synthetic TG as substrates and present our findings in this paper. A preliminary report on these data has been published (4).

MATERIALS AND METHODS

Gastric aspirates containing the lingual lipase were obtained as part of routine postnatal care at the Georgetown University Hospital. The samples were taken from infants whose gestational age was 33 to 42 weeks and the sample volumes were 0.5-5.0 ml. The samples were assayed for lipolytic activity and 32 with the highest levels were frozen on dry ice and sent to the University of Connecticut in a Styrofoam shipper. All arrived and were stored in a freezer at -75 C.

The digestion mixture was 0.1% citrate-Na_2HPO_4 buffer at pH 5.4, which contained 5% bovine plasma albumin and 0.1% gum arabic with a final volume of 25-50 ml. Prior to digestion, 2% of the desired TG was added, melted by heating on a steam bath if necessary and emulsified with a Branson Sonifier. The mixture was equilibrated at 37 C in a water bath and the gastric aspirate was added. The amount of aspirate used varied depending on the volume of the sample, but usually 2 equal portions were used, one for the TG and one for a control without substrate. Two samples were divided into 3 portions and both enantiomers

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Abbreviations: TG, triacylglycerol; DG, diacylglycerol; MG, monoacylglycerol; FFA, free fatty acids; *sn*-18:1-16:0-16:0, 1-oleoyl-2,3-dipalmitoyl-*sn*-glycerol; *sn*-16:0-16:0-18:1, 1,2-dipalmitoyl-3-oleoyl-*sn*-glycerol; and *sn*-16:0-18:1-18:1, 1-palmitoyl-2,3-dioleoyl-*sn*-glycerol.

were digested separately because of the relatively large volume. This procedure was necessary, as was the addition of an internal standard later, because each aspirate contained some lipid. We wanted to avoid the risk of denaturing the lipase by extraction of the aspirates with solvent. The length of incubation was 30-60 min depending on the activity of the aspirate.

The TG used as substrates were: *sn*-18:1-16:0-16:0, *sn*-16:0-16:0-18:1, *sn*-16:0-18:1-18:1 and trioleoylglycerol. The TG were synthesized as described by Jensen and Pitas (5).

After the desired period of incubation, the samples were extracted, the digestion products were separated by thin layer chromatography and the fatty acids were identified by gas liquid chromatography (GLC) after conversion to methyl esters (6,7). A known amount of methyl heptadecanoate was added to each fraction prior to analysis by GLC. The fatty acids in the aspirate controls were subtracted from each analysis of digestion product after equalizing the internal standards and adjusting the amounts of other acids accordingly.

To check our findings on stereospecificity, we determined specific rotations of the 1,2-(2,3) DG from a digestion of trioleoylglycerol. The *sn*-1,2 dioleoylglycerol has a specific rotation of -2.8 in $CHCl_3$ (8), which we confirmed with a standard. The specific rotation of the *sn*-2,3 enantiomer is +2.8. If a lipase is stereospecific, the optical rotation of DG formed by the enzyme would approach one or the other of these figures. A nonspecific lipase would produce a racemic mixture of DG with no optical rotation.

RESULTS AND DISCUSSION

The compositions of the original TG and the MG and FFA from the digestions of the TG are presented in Table 1. The preponderance of *sn*-3 acid in the FFA is clear evidence for the partial stereospecificity of lingual lipase—about 4:1 for the *sn*-3 ester. The composition of the MG was mostly the acid originally in the *sn*-2 position. The acid in the *sn*-3 position, whether 16:0 or 18:1, was hydrolyzed at equimolar rates. We calculated these data by reference to the internal standard. There was some digestion of *sn*-2 esters in contrast to pancreatic lipase which is very specific for the *sn*-1 and -3 positions.

Further confirmation of our data was provided by our determination of the specific rotation of the DG produced by the lipase. The specific rotation of the 1,2-(2,3) DG recovered from the digestion of trioleoylglycerol was -2.8°. The configuration of the DG was therefore almost totally *sn*-1,2 as the reported rotation is -2.8° (8). This overlooked method for the determination of lipase specificity has the advantages of being nondestructive and simple. The disadvantage is lack of sensitivity. The specific rotation of either enantiomeric DG is so small that dilution with the antipode would obliterate the difference between 2.8° and zero. Preparation of a derivative would increase the specific rotation and improve the sensitivity.

Stereospecificity is a novel characteristic in glycerol ester hydrolases. The property has been observed only in various serum stimulated lipoprotein lipases (9), rat lingual lipase (3) and hepatic lipoprotein lipase (10). Interestingly, the specificity of the lipoprotein lipases is partially for the *sn*-1 position. We cannot explain the physiological significance of the *sn*-3 specificity of the lingual lipases. However, we can postulate that the *sn*-1,2 DG are better substrates for pancreatic lipase than the enantiomers, although in vitro, the rates of digestion are influenced by the fatty acid composition of the DG. Morley et al. (9) noted that the dipalmitoyl DG accumulated when either

TABLE 1

Fatty Acid Composition of the Original Triacylglycerols, and the Monoacylglycerols and Free Fatty Acids Produced by Lipolysis with Premature Human Infant Lingual Lipase

Substrate and enzyme source	N	Triacylglycerol		Monoacylglycerol		Free fatty acids	
		16:0	18:1	16:0	18:1	16:0	18:1
Human		(M%)					
sn-16:0-16:0-18:1[a]	5	64.2	35.8	84.8	15.2	24.4	75.6
SEM		0.35		7.10		5.13	
sn-18:1-16:0-16:0	5	64.4	35.2	77.8	22.2	85.8	14.2
SEM		0.56		6.28		2.20	
sn-16:0-18:1-18:1	2	34.0	66.0	27.5	72.5	12.5	87.5

[a]1,2-Dipalmitoyl-3-oleoyl-*sn*-glycerol.

sn-16:0-16:0-18:1 or *sn*-18:1-16:0-16:0 was digested with pancreatic lipase. We can also postulate that the *sn*-1,2-DG act as messengers for receptor sites in the intestine, much as FFA stimulate the release of cholecystokinin from the intestinal wall (11). Another possibility might be related to the inability of the enzyme to hydrolyze the esterified fatty acids of *sn*-3 phosphatidylcholine, although the influence is not obvious (12). To summarize, the lipase in the gastric aspirates of newborn premature infants is partially stereospecific for *sn*-3 esters of TG.

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