Fat Digestion: Intestinal Lipolysis and Product Absorption

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Fat digestion in the breastfed newborn infant is a process catalyzed by three lipases. The process is initiated in stomach contents by gastric lipase and continues in the upper part of the small intestine by pancreatic colipase-dependent lipase and human milk bile-salt-stimulated lipase (BSSL).

Development of powerful techniques in molecular biology has made it possible to gain better insight into the structure of these lipases, which is necessary for a detailed understanding of the different functional aspects. We shall briefly discuss recent advances in structural knowledge of the lipases as well as their functional implications. We shall focus mainly on the human enzymes but, when relevant, also discuss corresponding enzymes of other species.

GASTRIC LIPASE

Gastric lipolysis and lipase activities of preduodenal origin have been recognized for many years. In humans the responsible enzyme is secreted by the chief cells of the gastric mucosa (1). The primary sequence of this 52-kDa glycoprotein is known through cloning and sequencing of cDNA (2). The tissue of origin differs between species but the amino acid sequence is highly conserved (2–5). Although, gastric lipase is of similar molecular size to colipase-dependent lipase the sequence shows only limited homology. Since gastric lipase is designed to act in the milieu of gastric contents, which inactivates colipase-dependent lipase, this is not surprising. With respect to structure-function relations a few characteristics have been described. Gastric lipase, unlike most other mammalian lipases, is dependent on a free sulphydryl group for activity (6). Like all other lipases gastric lipase must be able to interact with an emulsion surface. By removing the most N-terminal five amino acid residues by limited proteolysis we could show that an intact N-terminus is essential for lipid binding, and hence for activity against emulsified lipid substrates (Table 1) (7). A unique property of gastric lipase is its ability to initiate hydrolysis of a triglyceride.
emulsion with a surface coat of protein and phospholipid such as milk-fat globules (Table 2) (8). The structural explanation behind this is as yet unknown.

COLIPASE-DEPENDENT LIPASE

Pancreatic colipase-dependent lipase has long been regarded as the key enzyme in the digestion of dietary triglycerides (9), which constitute more than 95% of dietary lipids. Recently the primary structure of the human enzyme was revealed by means of cDNA cloning and sequencing (10,11). From these studies the molecular weight

### TABLE 2. Characteristics of the gastrointestinal lipases

<table>
<thead>
<tr>
<th></th>
<th>Gastric lipase</th>
<th>Colipase-dependent lipase</th>
<th>BSSL/CEH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site of action</td>
<td>Stomach</td>
<td>Small intestine</td>
<td>Small intestine</td>
</tr>
<tr>
<td>Cofactor</td>
<td>None</td>
<td>Colipase</td>
<td>Bile salt</td>
</tr>
<tr>
<td>Molecule size</td>
<td>52 kDa</td>
<td>50 kDa</td>
<td>107/100 kDa</td>
</tr>
<tr>
<td>Activity against human MFG*</td>
<td>110</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>End products*</td>
<td>FFA + DG</td>
<td>FFA + 2-MG</td>
<td>FFA + glycerol</td>
</tr>
<tr>
<td>Primary structure</td>
<td>Known</td>
<td>Known</td>
<td>Known</td>
</tr>
<tr>
<td>Crystal structure</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

* The activity of the lipases against human milk-fat globules (MFG) was tested under conditions optimal for each lipase. The values are expressed as percent of the activity against an artificial triolein emulsion emulsified with gum-arabic.

* FFA, free fatty acids; DG, diglycerides; MG, monoglycerides.

BSSL/CEH, bile-salt-stimulated lipase/carboxylic ester hydrolase.
of this glycoprotein was calculated to be around 50 kDa. A catalytically vital serine
residue, which is part of the consensus sequence for serine-hydrolases, G-X-S-X-G,
was verified. The complete gene structure, including the exon-intron arrangement,
has been determined for the canine enzyme (12). Comparison of the colipase-depen-
dent lipase gene with the known gene structures of other lipases, e.g., lipoprotein
lipase, has revealed common features (13). A major breakthrough in understanding
lipase function was when the three-dimensional structure of human colipase-depen-
dent lipase became known from X-ray crystallography (11). The enzyme is composed
of two distinct domains: one larger, involving the N-terminal part of the protein, and
a smaller formed by the C-terminal part. The larger domain is structurally very similar
to some microbial lipases, e.g., *Rhizomucor miehei* lipase (14). This domain includes
the active site buried in the interior of the molecule. A very interesting structural
feature is the so-called flap region, which is formed by a stretch of amino acid residues
giving an α-helical structure arranged as a lid covering the entrance to the “active
site pocket” (11). By sequence homologies it has been postulated that such a flap
structure is also present in lipoprotein lipase, although this has not been confirmed.
It seems likely that this flap is involved in the surface activation of colipase-dependent
lipase, i.e., activity against emulsified substrates. The enzyme would thus undergo
a conformational change when bound to an emulsion droplet whereby the flap is slid
aside giving the substrate molecules access to the active site pocket. A challenging
hypothesis is that this is a structural property shared by all typical lipases.

Colipase-dependent lipase is dependent for activity on its cofactor, colipase, also
secreted from the pancreas (15). Colipase, a small 9-kDa protein stabilized by a high
number of disulfide bonds, is highly conserved between species. In the absence of
colipase the lipase does not operate with maximal efficiency, particularly if bile salts
or other amphiphilic substances are present, which is the case in duodenal contents
during the digestive phase. Many studies have been carried out focusing on the exact
function of colipase and the interplay between cofactor and lipase (15). Very recently
van Tilbeurgh *et al.* (16) were able to co-crystallize the complex, thus revealing
the exact positioning of the two proteins when bound in complex. Colipase binds
exclusively to the smaller C-terminal domain of the lipase. This binding does not,
however, induce any conformational change in the lipase (16). Thus a likely function
of colipase is to promote productive binding of the lipase at the substrate surface
even when the surface is covered by amphiphilic substances.

**BILE-SALT-STIMULATED LIPASE**

Milks from only a limited number of species, e.g., humans, gorillas, cats, and dogs,
contain a bile-salt-stimulated lipase (BSSL) (17,18). The physiological importance
of BSSL is evident from fat balance studies in newborn infants. Heat treatment of
human milk, which inactivates BSSL (19), reduces fat absorption by as much as one-
third in preterm infants (20,21). Furthermore, using a kitten model, Wang and co-
workers (22) found that supplementing a formula devoid of BSSL activity with puri-
fied BSSL increased the weight gain of the kittens significantly compared with kittens
fed unsupplemented formula. The weight gain in fact, became comparable to that of kittens fed raw cat milk, containing endogenous BSSL (22).

We have cloned and sequenced cDNA, covering the entire coding sequence of the BSSL gene (23). The deduced protein contains 722 amino acid residues, i.e., it is considerably larger than the other two lipases. Apart from the consensus sequence around the active site serine, there are no regions homologous to other well-characterized lipases, e.g., gastric lipase, colipase-dependent lipase, and lipoprotein lipase. Hence it has a unique structure within the mammalian lipase family. In contrast, the N-terminal part of the peptide shows striking homology to typical esterases such as acetylcholine esterase (23). A surprising finding was that the C-terminal half of the molecule consists of a number of almost identical proline-rich repeats (23). A sequence of 11 amino acid residues is repeated 16 times; eight of these repeats are identical, having the sequence GAPPVPPTGDS. The functional significance of this highly unusual structure is a challenging question. It is, however, tempting to speculate that this structure partly explains the broad substrate specificity of BSSL, which is unique among lipases. However, such speculation is partly contradicted by the observation that when there are fewer repeats, as seen in the rat pancreatic counterpart (see below), this does not seem to change the properties of the enzyme dramatically (24).

Although the protein is highly glycosylated (25), it contains only a single N-glycosylation site, located in the vicinity of the active site serine (23). This means that in addition to this possible N-linked sugar there are several O-linked sugars. These are located in the region of the repeats, which contain two to three serine/threonine residues each. A tentative model of BSSL is shown in Fig. 1. Recently the complete gene structure of BSSL was elucidated (26). An interesting finding was the existence of a pseudogene. If this gene can be transcribed and translated, the resulting protein cannot function as a lipase since vital parts, including the active site serine residue, are missing.

**CARBOXYLIC ESTER HYDROLASE**

BSSL is closely related to the carboxylic ester hydrolase (CEH) secreted from human pancreas (27). The two are known to share many functional properties as well as immunochemical reactivity. The only difference noted is a minor difference in apparent molecular weight, i.e., 107 kDa for BSSL and 100 kDa for CEH when
determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (27). The cDNA sequences coding for both proteins are identical, strongly indicating that they are indeed products of the same gene (23,26,28,29). The possibility that alternative splicing explains the size difference cannot be ruled out; there is a recent report showing that BSSL in milk can be of two different sizes, one of which may be missing one exon (30). The remaining possibility is that the difference between BSSL and CEH is due to differences in tissue-specific glycosylation. The functional significance of this, if any, is as yet unknown. In the upper part of small intestinal contents the two operate as a single enzyme, the major part of which originates in the milk (31). In the following discussion, “BSSL” refers to the combined activity.

DIGESTION OF MILK FAT

There are several reasons why the two-phase model of fat digestion and absorption typical for the human adult (32) may not be applicable to the newborn infant, particularly the breastfed infant. First, the postprandial concentrations of colipase-dependent lipase in duodenal contents are much lower in neonates, especially if they are born before term (33). Second, the intraluminal bile salt concentrations are also low during the neonatal period (34), possibly often lower than required for micelle formation. Third, the milk itself provides BSSL, which adds to the infant’s endogenous capacity for fat digestion.

It is difficult to assess the relative quantitative role of each lipase in overall triglyceride digestion during the neonatal period. This is partly due to maturation of liver and pancreatic functions with gestational and postnatal age (35). While some studies suggest that gastric lipase and gastric lipolysis may account for the major part of triglyceride digestion (36), other studies suggest that its physiological function is qualitative rather than quantitative. Only gastric lipase, and not colipase-dependent lipase or BSSL, in its proper environment (mimicked in vitro) can initiate hydrolysis of native human milk fat globules (4,8,37). Based on in vitro experiments using purified enzymes and cofactors and human milk-fat droplets as substrate, we have recently proposed a model of how these three enzymes interact to promote efficient fat digestion (37). The basic concept is that they are all important not just by adding to the overall lipolytic capacity but by their individual and specific properties.

If human milk-fat globules are pretreated with gastric lipase, resulting in hydrolysis of only 5% to 10% of the triglyceride ester bonds, this dramatically alters the accessibility of the remaining triglycerides for subsequent hydrolysis in the upper small intestine. The partially digested globules are readily cleaved by colipase-dependent lipase or BSSL. The combined action of gastric lipase and colipase-dependent lipase results in hydrolysis of about two-thirds of the total triglyceride ester bonds, giving rise chiefly to sn-2 monoglycerides and free fatty acids (FFA) (37). These two are considered the main products of triglycerol digestion in adults.

In contrast to colipase-dependent lipase, BSSL is a nonspecific lipase, and thus lacks positional specificity (38). It can, therefore, also hydrolyze sn-2 monoglyceride. The final products of triglyceride digestion catalyzed by BSSL will be FFA and free
FIG. 2. The sequential steps involved in digestion of human milk triglycerides (TG). FFA, free fatty acids; BS, intraluminal bile salt concentration; DG, diglycerides; MG, sn-2 monoglycerides; G, glycerol; solid lines, enzymatic processes; dashed lines, non-enzymatic processes (e.g., transport).

glycerol. However, BSSL does not only affect the product pattern; it also affects the initial rate of lipolysis, i.e., hydrolysis of tri- and diglycerides. The relative importance of these BSSL functions may be considered in terms of intraluminal bile salt concentrations. In vivo, it can be expected that monoglycerides and FFA will be removed from the site of lipolysis by micellar solubilization and transport. However, since the capacity of micellar transport depends on the intraluminal bile salt concentration this will be a rapid and efficient process only when this concentration is high (Fig. 2). If this is so, most monoglyceride will escape further hydrolysis, and BSSL will contribute to lipolysis chiefly by supporting colipase-dependent lipase in hydrolysis of tri- and diglycerides. If, on the other hand, the bile salt concentration is low, which may be the normal state in many newborn preterm infants, the monoglycerides will remain in the lumen for a longer time period and BSSL will complete lipolysis by also hydrolyzing the monoglycerides (Fig. 2).

Interestingly, it is known that under conditions of low intraluminal bile salt concentration FFAs are more readily absorbed than monoglycerides (39). We have shown that when bile salt concentrations are low, lipolysis products are dispersed to a greater extent as unilamellar vesicles rather than as mixed micelles (40). Formation of vesicles is favored by a high FFA-to-monoglyceride ratio (41). Coexistence of vesicles can explain how the products can be carried from the emulsion surface, where lipolysis occurs, to the mucosal surface for absorption. However, how the products penetrate the enterocyte is as yet largely unknown. It has recently been suggested that lipases may also be involved in this process.

FATE OF THE INTESTINAL LIPASES

There are several studies on the immunohistochemical localization of BSSL/CEH in the small intestine (42–45). The results obtained are partly conflicting, e.g., whether
or not the enzyme can be localized to Paneth cells. Gallo et al. (42) suggested that the mucosal immunoreactivity was of pancreatic origin, i.e., depending on CEH, rather than resulting from local synthesis. Furthermore, it has been suggested that both colipase-dependent lipase and CEH bind to specific receptors on the microvillous membrane with the same affinity (46,47). The nature of the proposed receptor(s) is a heparin or heparin-like molecule attached to the membrane. Binding to the receptor(s) would facilitate uptake of lipolysis products. Moreover, it was shown using a cell line (Caco-2) that CEH can bind to and even be taken up by these cells (48).

The fate of the internalized enzyme was either degradation or resecretion (48). It should be noted that, judged by immunohistochemistry, little enzyme is found associated with the microvillous membrane (42, and Vilaros et al., unpublished). Hence, it would be an oversimplification to claim that the importance of the binding is mainly to facilitate the uptake of fatty acids. In order to elucidate the nature of this receptor binding and its possible role for product absorption or enzyme uptake requires more detailed study of the transport and handling of the respective enzymes and lipolysis products.

ACKNOWLEDGMENTS

This study was supported by grants from Swedish Medical Research Council (19X-05708), the National Board for Technical Sciences, Astra Hässle AB, and the Medical Faculty, University of Umeå.

REFERENCES


**DISCUSSION**

*Dr. Schmitz:* You said that bile-salt–stimulated lipase was similar to carboxyl ester hydrolase; then you said that the gene was absolutely unique.

*Dr. Hernell:* We cloned the BSSL cDNA and also part of the cDNA sequence of human pancreatic carboxyl ester hydrolase, and the deduced peptide chains are identical. However, there is still a difference in molecular size between the two. The most likely explanation for this is tissue specific glycosylation.

*Dr. Salle:* Why does BSSL exist only in primates?

*Dr. Hernell:* This is an interesting question. The enzyme is present in species other than primates, for example cat, dog, and ferret. It is not present in all primates, e.g., rhesus monkey, but is found in the African green monkey and the gorilla as well as in man. Caution is needed about earlier studies because they relied on activity measurements. They should now be reevaluated, looking for messenger RNA in the respective mammary glands. In suckling rat, carboxyl ester hydrolase, the pancreatic counterpart of BSSL, is the dominant lipase. It is predominant over the colipase-dependent lipases. It is only after weaning that colipase-dependent lipase becomes the dominant lipase. It may be that in some species there is a comparatively high activity in the pancreas, and there is no need for secretion of the lipase with the milk.

*Dr. Vidailhet:* What is the efficacy of gastric lipase in conditions such as cystic fibrosis where pancreatic insufficiency occurs?

*Dr. Hernell:* In cystic fibrosis and other causes of pancreatic insufficiency gastric lipase becomes the dominant lipase. No one has yet shown how much it contributes to digestion under these conditions. We know, however, that a substantial amount of fat is digested in cystic fibrosis (CF). In CF patients with pancreatic insufficiency, conditions in the intestine favor gastric lipase activity because there is much less protease activity and also lower pH. Therefore, gastric lipase may contribute to fat digestion not only in the stomach but also in intestinal contents.