Hydrolysis of human milk fat globules by pancreatic lipase: role of colipase, phospholipase A2, and bile salts.

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Research Article

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Hydrolysis of Human Milk Fat Globules by Pancreatic Lipase

ROLE OF COLIPASE, PHOSPHOLIPASE A_2, AND BILE SALTS

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ABSTRACT Human milk fat globules were used to explore how dietary triglycerides are hydrolyzed by pancreatic lipase. These triglycerides were hydrolyzed very slowly by lipase alone as if the surface layer of proteins and phospholipids impeded the action of the enzyme. The inhibition of lipase activity could be overcome by addition either of colipase or of pancreatic phospholipase A_2. Colipase enhanced triglyceride hydrolysis in a dose-dependent manner whether bile salts were present or not. Bile salts had no effect on the activity of pancreatic lipase alone but further enhanced the activity at all concentrations of colipase tested. Bile salts were a prerequisite to relieve inhibition of lipase activity by phospholipase A_2. Human milk fat globules exposed to phospholipase A_2 should be representative of a physiological substrate for pancreatic lipase. A major new observation was that bile salts, even at high concentrations, stimulated triglyceride hydrolysis of such phospholipase-treated globules by pancreatic lipase also in the absence of colipase.

INTRODUCTION

The present view on lipid digestion is based mainly on in vitro studies designed to probe the mode of action of pancreatic lipase and its interaction with colipase and bile salts. For these experiments simple model systems, such as with short-chain triglycerides (1–3) or lipid monolayers have generally been used. We recently studied the activity of pancreatic lipase against emulsions of longchain triglycerides covered with proteins or phospholipids (9). The results obtained deviated in several respects from the conclusions drawn from the model systems. We found, for instance, that colipase enhanced triglyceride hydrolysis also in the absence of bile salts (9). This prompted us to continue our studies with a natural substrate. Reflecting the interest of our group, we chose to use human milk fat globules, the main nutrient of the breast-fed newborn. From model studies it has been concluded that bile salts displace pancreatic lipase from interfaces, thereby inhibiting its activity, and that colipase under such conditions provides an anchor for the binding of lipase. Thus, colipase is a prerequisite for activity of lipase in the presence of bile salts (2, 3, 6). In contrast, we found that with milk fat globules that had been exposed to phospholipase A_2 bile salts enhanced rather than inhibited lipase activity in the absence of colipase.

METHODS

Purified porcine pancreatic lipase (10) and colipase (11) were generous gifts of Dr. R. Verger (Marseilles, France). The pancreatic lipase showed a single band on sodium dodecyl sulfate gel electrophoresis and had a specific activity of ~650,000 nmol fatty acids released/min·mg (pH 7.5, trioleate/gum arabic emulsion). This is similar to that previously reported (9) and to that obtained by Borgström (12) with Intralipid as substrate. Colipase was a mixture of colipase 1 and 2 in their active, trypsinated forms. Triolein labeled with [^{3}H]oleic acid and sodium taurocholate[1] were kindly prepared by Dr. L. Krabisch (Lund, Sweden). Triolein was purified by thin-layer chromatography before use. Porcine pancreatic phospholipase A_2 and gum arabic were from Sigma Chemical Co. (St. Louis, Mo.). Human milk was collected by a breast-pump from mothers during their 1st wk of lactation. It was stored at 4°C. Preparation of the milk fat globules was started within a few hours by first heating the milk to 60°C for 15 min. Such heating completely inactivated both the bile salt-stimulated lipase activity (13, 14) and the lipoprotein lipase activity (15). The protein pattern of milk fat globules on sodium dodecyl sulfate polyacrylamide gels was not altered by the heating, which indicates no major disturbance

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[1] *Abbreviation used in this paper*: NaTC, sodium taurocholate.

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of the membranes. After heating, the milk was centrifuged at 10,000 *g* for 20 min. The tubes were sliced and the cream was washed twice with 0.15 M NaCl. Finally, it was resuspended in 40% of the initial volume in 0.15 M NaCl.

The typical incubation mixture contained 40 μl of isolated milk fat globules. It was 0.2 M in Tris-maleate buffer, pH 6.5, 20 mM in $CaCl_2$, and 0.15 M in NaCl. The triglyceride concentration was assumed to be 4,500 nmol/ml incubation mixture based on a concentration of 4% fat in whole milk. The total incubation volume was 1 ml. Incubations were carried out at 37°C in a water bath shaking at 50 strokes/min. Details of the individual incubations are specified in figure legends.

The fatty acids released were extracted and titrated (16). Palmitic acid was used as a standard. Trioleate - gum arabic emulsions were prepared as described (9). The triglyceride content was 4,500 nmol/ml, and the total incubation volume was 0.2 ml. Lipase activity is expressed as nanomoles fatty acid released per milliliter of incubation mixture.

RESULTS

When pancreatic lipase was added to a medium that contained only milk fat globules, Ca^{++}, and buffer, there was little or no hydrolysis of the triglycerides. In contrast, under otherwise identical conditions, the lipase rapidly hydrolyzed an emulsion of trioleate in gum arabic (Fig. 1). The slow hydrolysis of milk triglycerides was probably due to inhibition caused by the milk fat globule membrane (9), i.e., the layer of protein and phospholipids that envelopes the triglyceride core (17).

Effect of colipase and bile salts. The inhibition could, at least partly, be overcome by addition of colipase to the medium containing milk fat globules and lipase. Bile salt had no effect on the activity of lipase alone but enhanced the activity in the presence of colipase (Fig. 2). Similar data were obtained when heat-treated whole human milk rather than isolated milk fat globules was used as substrate (data not shown). The bile salt effect is demonstrated in Fig. 2 with 1.5 mM NaTC. This concentration was chosen because it is physiologically relevant considering the situation in the small intestine of the newborn infant (18–19). Both at low and high concentrations of colipase the rate of hydrolysis increased with increasing bile salt concentrations, at least up to a concentration of 20 mM (Fig. 3*a*). Colipase enhanced triglyceride hydrolysis in a dose-dependent manner whether bile salts were present or not (Fig. 3*b*), but at all concentrations of colipase used (at most 50 μg/ml) the rate of hydrolysis was always further enhanced by the addition of bile salts.

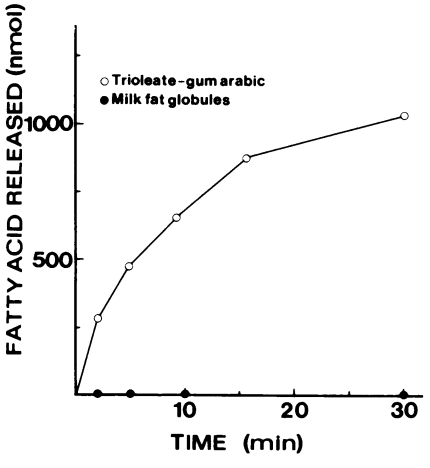

FIGURE 1 Hydrolysis of milk fat globules and of long-chain triglycerides emulsified in gum arabic by pancreatic lipase. Conditions were as described in Methods. Pancreactic lipase (0.25 μg/ml) was added at start to either milk fat globules or to an emulsion of trioleate in gum arabic. The fatty acids released were determined titrimetrically and by measuring radioactivity, respectively. The values obtained are expressed per milliliter of incubation mixture for comparison.

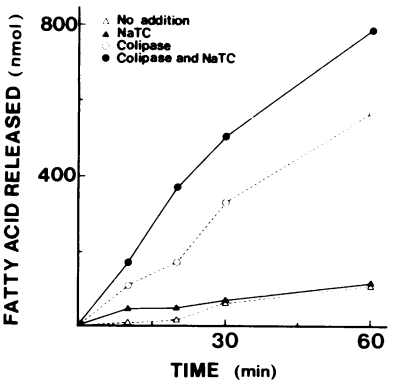

FIGURE 2 Effect of colipase and/or NaTC on the hydrolysis of milk fat globules by pancreatic lipase. Conditions were as in Methods. 0.15 μg/ml lipase was added at start. As indicated, 1.5 mM NaTC and/or colipase (40 μg/ml) was added 10 min before the lipase.

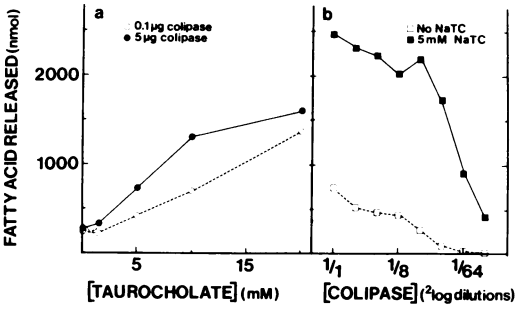

FIGURE 3 Colipase enhancement of the hydrolysis of milk fat globule triglyceride by pancreatic lipase. Effect of increasing amounts of NaTC or colipase. Conditions were as in Methods. In *a* either 5 μg/ml or 0.1 μg/ml of colipase was added 10 min before the lipase (0.3 μg/ml). The concentrations of NaTC varied. In *b* the amount of colipase was varied from 5 μg (1/1) to 0.04 μg/ml (1/128). It was added 10 min before the lipase (0.6 μg/ml). The incubations were performed either in the presence or in the absence of 5 mM NaTC. The amounts of fatty acids released represents the 10-min value from a time curve for each concentration.

Effect of phospholipase A_2 and bile salts. When an excess of phospholipase A_2 was added, either before or together with the lipase, hydrolysis of the triglycerides started immediately, provided bile salts were also present. When either bile salts or phospholipase was not present during the first part of the incubation but was added 20 min after the lipase, the initial hydrolysis was again slow, but when the system became complete, the rate of hydrolysis immediately became as rapid as when both phospholipase and bile salt were included at the start (Fig. 4). Thus, the low rate of hydrolysis was not due to inactivation of the lipase.

At lower phospholipase concentrations, i.e., <1 μg/ml, the rate of triglyceride hydrolysis increased with time during the first 5–10 min (data not shown). This again demonstrated the necessity of phospholipid hydrolysis before efficient triglyceride hydrolysis. In the system with an excess of phospholipase the rate of triglyceride hydrolysis increased with increasing bile salt concentration, at least up to a concentration of 10 mM (Fig. 5). Thus, in spite of the fact that the system did not contain colipase there was no evidence of loss of activity owing to desorption of the lipase from the oil-water interface by bile salts.

DISCUSSION

The experimental conditions were designed to resemble the physiological situation. The pH was generally 6.5 and the substrate used was isolated human milk fat globules. The concentrations of bile salts, colipase, and phospholipase A_2 were varied. The amount of lipase was chosen for experimental convenience but was within the range found in the intestine of the newborn (20–21). The principal results were the same independent of the lipase concentration used. Under conditions found optimal for hydrolysis

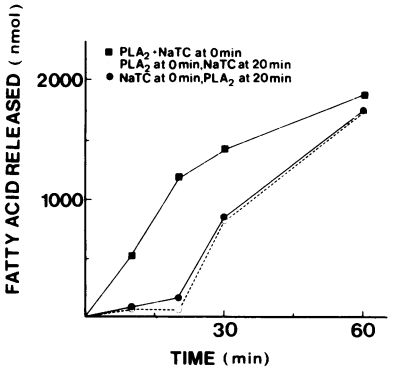

FIGURE 4 Effect of phospholipase A_2 and NaTC on the hydrolysis of milk fat globules by pancreatic lipase. In all experiments 0.3 μg/ml of lipase was added at start. Phospholipase A_2 (10 μg/ml) was added either at start (■, ○) or after 20 min (●). NaTC (5 mM) was included at start (■, ●) or added after 20 min (○).

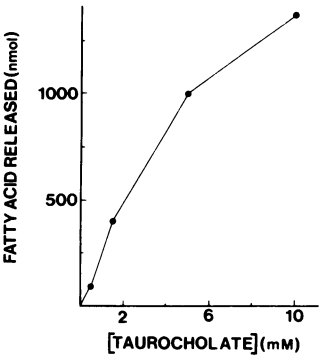

FIGURE 5 Effect of NaTC concentration on the enhancement of pancreatic lipase hydrolysis of milk fat globules by phospholipase A_2. Pancreatic lipase (0.3 μg/ml) was added at start; phospholipase A_2 (10 μg/ml) and NaTC were added 2 min before the lipase. For each NaTC concentration a time curve was made. The values in the figure represents the amount of fatty acid released after 20 min from each curve.

of the milk fat globules, the turnover number of the enzyme was similar to that obtained with other emulsions of long-chain triglycerides (9, 12).

When purified pancreatic lipase was incubated with isolated milk fat globules virtually no release of fatty acids could be detected, at least for the time period tested, i.e., up to 2 h. The milk fat globule consists of a core of triglycerides enveloped by a surface layer of proteins and phospholipids (17). The same is probably true for most dietary lipids when presented to the digestive enzymes. We, as well as others, have previously shown that artificial emulsions of triglycerides with a surface cover of proteins (9, 22, 23) or phospholipids (9, 24) are poor substrates for pancreatic lipase.

This study demonstrates that an interplay of many factors promotes a rapid and complete hydrolysis of the milk fat globule triglycerides. There are at least two principal mechanisms by which the apparent inhibition of pancreatic lipase can be relieved: first, by adding colipase, and the more colipase added the higher rate of hydrolysis, up to a certain limit. This confirms our previous observation with artificial long-chain triglyceride emulsions covered with proteins or phospholipids (9). The second mechanism is by exposing the substrate to phospholipase A_2 and bile salts. The effect of bile salts could possibly be explained by an effect on phospholipid hydrolysis (25) and/or by a desorption of the lysophospholipids formed as a result of the action of phospholipase (9). The major new finding in this study, however, is that regardless of which of the two principal mechanisms by which pancreatic lipase activity is restored, bile salts, also in very high concentrations, i.e., 10–20 mM NaTC, stimulate triglyceride hydrolysis. In other words, bile salts enhanced hydrolysis both in the presence and in the absence of colipase.

In the latter case, however, bile salts only had effect when phospholipase A_2 was also present.

Borgström recently reported (12) that when lipase acted on Intralipid, an emulsion of long-chain triglycerides stabilized by phospholipids, there was a lag phase during which hydrolysis was very slow. The fatty acids released by lipolysis relieved the apparent inhibition, so that ultimately a high rate was attained. Under conditions chosen to bring out the lag time it was >1 h. These conditions, however, were not physiological. In our experiments we did not detect any lag time. This is, in fact, in agreement with Borgström, who found that the lag time is highly pH dependent and is only a few minutes in the pH range 6–7. Even under conditions when we found very low rates of hydrolysis these did not increase with time. In our experiments hydrolysis was either linear with time or decreased with time. The only exception was in a system with bile salts and suboptimal concentrations of phospholipase but without colipase, where the rate of triglyceride hydrolysis initially increased with time. Addition of free fatty acids to the incubations had no effect on the rate of hydrolysis. In fact, this is also in agreement with Borgström (12) who found that fatty acids reduced the lag time but did not influence the maximal velocity obtained after the lag time.

The general view of the role of colipase is that it overcomes the inhibition of lipase by bile salts. Milk fat globules exposed to phospholipase A_2 should be representative of a type of substrate on which pancreatic lipase acts in the intestine. Interestingly, bile salts enhanced rather than inhibited the triglyceride hydrolysis of such phospholipase-treated milk fat globules by lipase. Thus, the inhibition which colipase was supposed to overcome did not exist. Furthermore, colipase enhanced the hydrolysis even when the system did not include any bile salt. In these systems the action of colipase and bile salts was synergistic, rather than opposite. This raises the question whether the sole effect of colipase is to allow lipase to bind to its substrates or whether it also promotes the catalytic efficiency of the lipase at the interface. Such an effect would be analogous to that of apolipoprotein CII on lipoprotein lipase (26). In fact, a three-to-fourfold increase by colipase on the catalytic efficiency of lipase has been demonstrated on a mixed monomolecular film of triglycerides/lecithin (27).

Healthy adults have a high capacity to digest and absorb lipids. The present study demonstrates that several factors, i.e., pancreatic lipase, colipase, pancreatic phospholipase A_2, and intraduodenal bile salts together promote an efficient hydrolysis of dietary lipids. However, neither colipase nor phospholipase nor bile salts seem to be indespensible for lipolysis. For instance, under conditions of low concentrations or deficiency of bile salts the inhibition of pancreatic lipase can be relieved by colipase alone (9) (Fig. 2). Indeed, isolated deficiencies in one of the factors are compatible with absorption of a substantial amount of lipids (28–31). Newborn infants, especially preterm infants, have a reduced capacity to hydrolyze dietary lipids (32) and they probably have low levels of all the factors involved. Their intraduodenal concentrations of pancreatic lipase (20–21) and bile salts (18) are low. The general immaturity of the pancreas (33) taken together with the observation that colipase and lipase activities vary in parallel (21), makes it also likely that colipase and phospholipase concentrations are low. This may explain why neonates probably are dependent on other factors such as the lingual lipase (34) and the bile salt-stimulated lipase in human milk (35) for optimal lipid digestion.

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