Properties of Intestinal Lipase*

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Little is known regarding intestinal lipase, although its participation in the absorption of neutral fat from the gastrointestinal tract has been suggested (1). Lipolysis by intestinal enzymes was first established by Schiff (2) in studies with depancreatized dogs. Kalaboukoff and Terroine (3) later reported that glycerol esters of intestinal mucosa hydrolyzed olive oil and that this activity was accelerated by the addition of bile salts.

In this study, the lipase activity of intestinal mucosa has been examined. In order to limit the study to lipase activity, we have employed long chain triglycerides as substrate in the form of olive oil emulsions. As will be seen, the intestine also contains acyltransferases, as judged by the rapid hydrolysis of short chain fatty acid esters. These enzymes would not be expected to interfere with lipase estimations, since, in other tissues such as the liver, they have been shown to be completely inactive toward long chain triglycerides.

The pancreatic enzyme has generally been regarded as the prototype of lipases. We have therefore compared the intestinal and pancreatic enzymes with regard to substrate specificity and the effects of various activators and inhibitors, a number of which have been employed by other investigators to characterize esterolytic enzymes. Although the intestinal activity appears to be similar, in general, to that of the pancreas, it is clear that the enzymes involved are not identical.

The possible participation of intestinal lipase in the intestinal absorption of fat is discussed.

EXPERIMENTAL PROCEDURE

Materials and Methods

Preparation of Substrate—For routine assay of lipase activity, olive oil was employed as substrate. A stock emulsion containing 10% olive oil, 0.5% mono-fatty acid ester of polyethylene glycol ("Ethenol" C/15, Armour Chemical Division), 0.5% purified soybean phosphatides (American Lecithin Company, Inc.), 0.25% sorbitan monolauroate ("Span 20," Atlas Powder Company), 0.10% sodium cholate, 0.50% polyglycerol ester of fatty acids ("Demol 14," Emulsol Corporation), and 5.0% glucose was prepared in a dairy homogenizer (4). The emulsifiers did not contribute significantly to the optical density of the reaction mixture and were not hydrolyzed by the enzyme preparations.

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This stock emulsion contains 0.34g meq of esterified fatty acids contributed by olive oil per milliliter; this is calculated with 277 as the average molecular weight of fatty acids of olive oil. The emulsion remained stable throughout the reaction.

1-Monolein (98% pure) and 1,3-diolein (95% pure) were kindly supplied by Dr. L. A. Goldblatt of the Southern Utilization Research and Development Division, Agricultural Research Service, New Orleans, Louisiana.

Determination of Fatty Acids—Microequivalents of esterified fatty acids were determined by Stern and Shapiro's modification (5) of the hydroxylamine method of Lipmann and Tuttle (6, 7). Unesterified fatty acids were measured as follows. An equal volume of ethanol was added to the reaction mixture; the solution was adjusted to pH 10 to 11 with NaOH and extracted twice with petroleum ether to remove esterified fat. The combined petroleum ether extracts were then extracted with alcoholic ethano1, which was added to the original ethanolic solution. The latter was acidified with HCl and the fatty acids liberated were extracted twice with petroleum ether. The extracts were combined and washed twice with 10% ethanol and evaporated to dryness under nitrogen. The isolated fatty acids were dissolved in ethanol, heated to 65°, and the solution titrated with aqueous 0.05 N NaOH, with phenolphthalein as an indicator. The ethanol concentration in the titration flask was never permitted to fall below 50%.

Assay System—As shown in Fig. 1, the liberation of free fatty acids was proportional to the clearing of the emulsified substrate as measured by the decrease in optical density at 700 mp. The optical method proved to be most convenient for routine assay. The procedure was as follows. Into a 3-ml Beckman cuvette were pipetted 2.60 ml of Tris buffer (0.10 M, pH 9.0), 0.20 ml of a 1:10 dilution of the stock emulsion in the same buffer, and 0.20 ml of enzyme solution. This amount of substrate gave a linear reaction rate spectrophotometrically until approximately 70% of the ester was hydrolyzed. The total reaction was run for 60 minutes, during which time the optical density decreased from an initial 0.70 to about 0.20.

A unit of enzyme was defined as that amount which produced a decrease of 0.001 optical density units per minute.

Preparation of Cell-free Duodenal Homogenate—In a preliminary survey, the lipase activity of mucosal homogenates of 5 species of animals was tested. The order of decreasing activity was as follows: rat, hog, dog, rabbit, and cow. For further study, the hog intestine was chosen because of its availability in large quantity. Approximately the first 12 inches of hog duodenum were removed within about 10 minutes after the slaughter of the animal. Each segment was split longitudinally, washed twice in water, and placed on dry ice. All subsequent steps were carried out at 0-2° unless otherwise stated. After thawing, the
duodenal strips were washed 7 to 8 times in large quantities of tap water and then 2 to 3 times with distilled water.

Approximately 400 g of tissue were blotted as free of water as possible and finely ground in an electric meat grinder. The resulting pulp was homogenized in a Waring Blender for 1.5 minutes with 4 ml of water per g of tissue. Because of its high viscosity, it was necessary to centrifuge at 3,500 X g for 20 minutes to sediment the nuclei and cellular debris. The supernatant containing the mitochondria and microsomes, after removal of the white lipid scum, was stored at -20°. This fraction is referred to as the duodenal homogenate; its protein content was determined to be approximately 15 mg per ml by the method of Gornall et al. (8), modified to give a final NaOH concentration of 0.8 N.

Effects of Enzyme and Substrate Concentrations on Lipase Activity—As shown in Fig. 2, the rate of lipolysis was proportional to the concentration of enzyme tested. The enzyme preparation employed was a cell-free homogenate of hog duodenum and contained 14.6 mg of protein per ml. Quantities of this homogenate ranging from 0.59 to 2.92 mg were employed in the usual 3 ml assay system. The relationship of substrate concentration to enzyme activity is shown in Fig. 3. A Lineweaver-Burk plot of the same data produces a linear relationship with half maximal activity observed at 3.0 x 10⁻⁴ moles per liter. The validity of calculating an affinity coefficient is of questionable value in view of the uncertainty regarding the physical properties of emulsions, especially particle size.

Localization and Solubilization of Enzyme Activity—The specific activities of the mitochondrial and microsomal fractions of duodenal homogenate were found to be 11 and 14 times higher than that of the soluble fraction (Table I). In order to obtain satisfactory differential centrifugation it was first found necessary to sediment all particulate matter out of the above described viscous duodenal homogenate by centrifuging twice at 25,000 X g for 60 minutes. The pellet was then resuspended in 0.25 M sucrose, after which the nuclear, mitochondrial, and microsomal fractions were separated by conventional methods of differential centrifugation (9).

The particle-bound activity of the duodenal homogenate could be solubilized at alkaline pH (Fig. 4). A 2:1 dilution of blood serum or bovine serum albumin (0.5%) was similarly effective in the pH range 6.5 to 7.5. Egg albumin, a proteose-peptone mixture of beef heart, or heparin in concentrations ranging from 0.25 to 1.5 mg per ml were ineffective.

Stability of Intestinal Preparation The lipase activity of the duodenal homogenate was not reduced after incubation for 24 hours at 37° at pH 7.0. Below pH 5 and above pH 10, a significant loss of activity occurred within 10 minutes. Heating a soluble preparation at neutral pH to 55° for 4 minutes caused no inactivation; at 60°, however, the activity was completely de-

![Fig. 2. Relationship between lipase activity and enzyme concentration. Lipolytic activity was assayed by the rate of clearing as described under "Experimental Procedure."](image)

![Fig. 3. Effect of substrate concentration on enzymatic activity. Experimental conditions were as described for the routine assay system except for the varying concentration of emulsion employed. Molarity of olive oil substrate was calculated on basis of triolein, molecular weight of 885.](image)

![Fig. 4. Solubilization of enzyme activity at alkaline pH.](image)

![Table I](image)

**Lipase activity of duodenal homogenate fractions**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Activity</th>
<th>Specific activity</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenal homogenate</td>
<td>423</td>
<td>1.5</td>
<td>100.0</td>
</tr>
<tr>
<td>Nuclei* (600 X g)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mitochondria (8,000 X g)</td>
<td>51</td>
<td>10.0</td>
<td>12.1</td>
</tr>
<tr>
<td>Microsomes (25,000 X g)</td>
<td>149</td>
<td>12.5</td>
<td>35.0</td>
</tr>
<tr>
<td>Soluble (25,000 X g)</td>
<td>219</td>
<td>0.9</td>
<td>51.8</td>
</tr>
<tr>
<td>Sum of fractions</td>
<td>419</td>
<td>-</td>
<td>98.9</td>
</tr>
</tbody>
</table>

*This fraction is the small amount of nuclear material not removed by the preliminary centrifugation of the original homogenate (see "Experimental Procedure").
The particles were resedimented by centrifugation at 25,000 × g for 60 minutes and 0.2 ml of each supernatant was assayed for its clearing activity. The pH of the mixtures remained that of the initial buffers. The pH of every vessel was checked at the beginning and end of the experiment and found to be unchanged.

The preparation was stable to 90% acetone at room temperature for at least 10 minutes and could be frozen and stored at −20°C for several months without loss of activity.

**Effect of pH and Temperature**—As shown in Fig. 5, the optimal pH for intestinal lipase activity was about 9. The apparent absence of activity below pH 7 was an artefact due to the formation of insoluble free fatty acids which masked clearing in the assay system. This could be shown by the fact that some clearing was demonstrable below pH 7 when 0.7% serum albumin was present to remove free fatty acids by complex formation.

The temperature coefficients for lipolysis were 2.0 in the range of 8–18°C, 1.5 between 18–28°C and 1.2 between 28–38°C. 

**Effects of Various Activators and Inhibitors**—Table II shows the effects of a number of activators and inhibitors on the lipase activities of intestinal and pancreatic preparations.

1. A cofactor requirement for the soluble fraction of intestinal homogenate could not be demonstrated. The active material was precipitated in toto by adjusting the solution to 30% saturation (21 g/100 ml) with ammonium sulfate and to 0.20 M with sulfuric acid (11). The supernatant was discarded and the redissolved precipitate was found to be almost fully active. Dialysis for 24 hours against 0.02 M Tris buffer (pH 6.0) and 0.01 M ethylenediaminetetraacetic acid did not cause any significant loss of activity. By contrast, pancreatic lipase was inactivated by 18 hours of dialysis with or without EDTA.1

2. Fluoride did not inhibit intestinal or pancreatic lipase activities. Hollett and Meng (12) also failed to show fluoride inhibition of the pancreatic enzyme at the concentration employed in this study, although this has been reported by others (13). This discrepancy may be explained by the observation that fluoride will partially inhibit the stimulatory effect of protein on pancreatic lipase. Thus, we have observed a 25% inhibition by 2.5 × 10−3 M fluoride when the pancreatic enzyme was tested in the presence of 1% serum albumin or as a crude homogenate.

3. Intestinal lipase activity was completely inhibited by 2 × 10−3 M p-chloromercuribenzoate. This inhibition was readily reversed by 4 × 10−3 M cysteine. PCMB, 2 × 10−4 M, had no effect. Pancreatic lipase was only transiently inhibited by PCMB under the same conditions; after 5 to 10 minutes the clearing reaction resumed at about 60% of the control rate and was not inhibited when the PCMB concentration was then doubled.

4. Aldridge (14) has differentiated lipases from esterases on the ground that the latter are inhibited by organophosphorus compounds, such as diethyl p-nitrophenyl phosphate (E600) in concentrations as low as 1 × 10−4 M. Intestinal lipase activity was found to be relatively insensitive to this substance, in fact even less sensitive than pancreatic lipase. The intestinal preparation was also insensitive to eserine and atoxyl which are potent esterase inhibitors.

5. Quinine has also been employed to distinguish lipases, which are inhibited by this substance, from esterases, which are unaffected (15). Intestinal lipase activity was reduced by 50% in the presence of 1 × 10−3 M quinine. Pancreatic lipase was equally inhibited by about 1/5 of this concentration under the same conditions. Similarly, the intestinal homogenate could be inhibited to a lesser extent than pancreatic lipase by acetonitrile and benzaldehyde. Sodium glycocholate (1 × 10−3 M), which has been reported to activate pancreatic lipase (16), had little or no effect in our hands on either the intestinal or pancreatic enzymes.

6. Calcium ions (8.0 × 10−4 M) produced a considerable stimul-
lination of lipolysis, but this effect probably was due to the removal of liberated fatty acids through the formation of insoluble calcium soaps. In order to demonstrate the rate of lipolysis in this experiment, EDTA was added to aliquots removed at intervals from the reaction mixture and the optical density of each aliquot was determined. The rate of lipolysis of the reaction mixture containing calcium was found to be 174% that of a similar mixture containing no added calcium. Free fatty acids are known to be inhibitory to pancreatic lipase.

7. Purified bovine serum albumin inhibited lipolysis by the intestinal enzyme in the alkaline pH range (Table II and Fig. 5). Under the same conditions, at pH 9.0, pancreatic lipase was stimulated approximately 5-fold.

Possible Participation of More Than One Protein Component in

**TABLE II**

Effects of various activators and inhibitors on intestinal and pancreatic lipases

A control was run concurrently in each instance, omitting only the agent or particular condition under test. The source of intestinal lipase was the cell-free duodenal homogenate described under "Experimental Procedure." The pancreatic preparation was "Steapsin" (Nutritional Biochemicals Corporation), prepared as a 1% solution in water, except in the test with albumin in which case a fresh pancreatic homogenate, diluted 36:1 with water, was employed; the volume of either pancreatic preparation added to the test system was 0.10 ml. Activity was assayed by the decrease in turbidity except in the case of quinine and calcium which interfere with the optical method. In these instances lipolysis was determined chemically by the liberation of fatty acids (see "Experimental Procedure"). The abbreviations and other details are explained in the text.

<table>
<thead>
<tr>
<th>Lipase tested</th>
<th>Agents and conditions</th>
<th>Rate of hydrolysis</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestinal</td>
<td>Dialysis</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>EDTA (10^{-3} M)</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>Dialysis + EDTA (10^{-3} M)</td>
<td>82</td>
<td>82</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>Dialysis</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>EDTA (10^{-2} M)</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Dialysis + EDTA (10^{-2} M)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Intestinal</td>
<td>Fluoride, 2.5 × 10^{-3} M</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>Fluoride, 2.5 × 10^{-3} M</td>
<td>97</td>
<td>97</td>
</tr>
<tr>
<td>Intestinal</td>
<td>PCMB 2 × 10^{-3} M</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PCMB 2 × 10^{-3} M + Cysteine 4 × 10^{-3} M</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Intestinal</td>
<td>E600, 0.4 × 10^{-4} M</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>E600, 1.3 × 10^{-4} M</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>E600, 2.0 × 10^{-4} M</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>E600, 0.4 × 10^{-5} M</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>E600, 1.3 × 10^{-5} M</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Intestinal</td>
<td>Quinine 1.5 × 10^{-3} M</td>
<td>43</td>
<td>43</td>
</tr>
<tr>
<td>Intestinal</td>
<td>Ca^{2+} 8.0 × 10^{-4} M</td>
<td>174</td>
<td>174</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>Ca^{2+} 0.8 × 10^{-4} M</td>
<td>173</td>
<td>173</td>
</tr>
<tr>
<td>Intestinal</td>
<td>Serum albumin 0.7%</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>Serum albumin 0.7%</td>
<td>536</td>
<td>536</td>
</tr>
</tbody>
</table>
In an attempt to investigate the aliesterase and lipase contents of the intestinal homogenate, the preparation was assayed by the method of Seligman and Nachlas (18) as seen in Table III. The clearing activity toward olive oil emulsion is shown for comparison. The Seligman and Nachlas method is based upon the proposal that aliesterase but not lipase hydrolyzes \( \beta \)-naphthyl laurate in the absence of bile salt. In the presence of bile salt, on the other hand, lipase is stimulated to hydrolyze \( \beta \)-naphthyl laurate, whereas aliesterase activity is partially inhibited. Pancreas was found to be very rich in both aliesterase and “lipase” according to these criteria and was also very active in the clearing of olive oil emulsion. The liver had 60\% of the aliesterase activity of the pancreas per milligram of protein, but was completely lacking in “lipase” and clearing activities. Homogenates of kidney, also known to be high in aliesterase content, similarly failed to clear olive oil. The duodenal preparation showed twice as much aliesterase as “lipase” activity and the specific activities were relatively low. As judged by this test, there was no longer the marked preponderance of aliesterase activity which was suggested by the data of Fig. 8. Furthermore, the suggestion that \( \beta \)-naphthyl laurate may be a poor substrate for the intestinal lipase receives some support in the observation (Table III) that the ratio of clearing to lipase activity was about 4:1 for the duodenal preparation whereas it was nearly 1:1 for the pancreatic preparation.

**DISCUSSION**

Because of the relatively large mass of the mucosa, the total lipase activity of the small intestine may approach that of the pancreas, although the specific activity of the intestinal homogenate would appear most probable that the intestinal preparation is grossly contaminated with aliesterases. The question arises, however, as to why the activity toward long chain \( \beta \)-naphthyl esters is apparently so low whereas activity toward long chain triglycerides is readily demonstrable. It is possible that the long chain \( \beta \)-naphthyl derivatives are poor substrates for the intestinal enzyme.

**TABLE III**

Aliesterase and lipase activities assayed by Seligman-Nachlas procedure compared to clearing activity toward olive oil emulsion

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Aliesterase (units/mg protein)</th>
<th>Lipase (units/mg protein)</th>
<th>Clearing (units/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreas</td>
<td>292</td>
<td>209</td>
<td>221</td>
</tr>
<tr>
<td>Liver</td>
<td>194</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Duodenum</td>
<td>6</td>
<td>3</td>
<td>11</td>
</tr>
</tbody>
</table>

* Units of aliesterase and lipase are as defined by Seligman and Nachlas, whereas units of clearing are those defined under “Assay System” in the present work.

Pancreatic and liver homogenates were prepared as described for the experiment shown in Fig. 8. Aliesterase and lipase determinations were by the colorimetric method of Seligman and Nachlas (18) modified by incubation at 37\°C for 1 hour instead of 5. The amount of each sample was: (a) pancreas, 0.01 mg of protein; (b) liver, 0.01 mg of protein; (c) duodenum, 0.13 mg of protein. Clearing of olive oil was carried out in the standard way except for pancreas, where only 0.02 ml of the homogenate was used.
The intestinal lipase readily splits monoglycerides which, in our
further hydrolysis and resynthesis of triglycerides (20) occur
the cells of the intestinal wall. Numerous observations suggest
the principal action of intestinal lipase is not within the lumen of the gut but within
the pancreatic duct, presumably due to the lipase content of intes-
timate correlation between the quantity of enzyme available,
50% or more of ingested fat may undergo hydrolysis after ligation of the
the enzyme activity toward monolein indicate that the intestinal
hydrolysis and absorption of fat. It has been shown, in fact, that 50% or
more of ingested fat may undergo hydrolysis after ligation of the
pancreatic duct, presumably due to the lipase content of intestinal juice (1). It is probable, however, that the principal action
of intestinal lipase could play a significant role in the hydrolysis
preparation. Thus, in terms of the quantity of enzyme available,

The intestinal lipase is similar to Steapsin in that it is inhibited
by p-chloromercuribenzoate, diethyl-p-nitrophenyl phosphate, quinine, free fatty acid, and albumin. Little or no loss of activity was observed after dialy-
ysis with or without ethylenediaminetetraacetic acid. The addi-
tion of eserine, atoxyl, protamine, or fluoride produced no sig-
ificant inhibition of the enzyme. The effect of these agents
and the activity toward monolein indicate that the intestinal
enzyme is distinct from that of the pancreas.

3. The enzyme was inhibited by p-chloromercuribenzoate,
diethyl-p-nitrophenyl phosphate, quinine, free fatty acid, and albumin. Little or no loss of activity was observed after dialy-
ysis with or without ethylenediaminetetraacetic acid. The addi-
tion of eserine, atoxyl, protamine, or fluoride produced no sig-
ificant inhibition of the enzyme. The effect of these agents
and the activity toward monolein indicate that the intestinal
enzyme is distinct from that of the pancreas.

4. The possible role of intestinal lipase in fat absorption is
discussed.

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