PURIFICATION AND PROPERTIES OF AN ACID LIPASE FROM HUMAN GASTRIC JUICE

C. TIRUPPATHI and K.A. BALASUBRAMANIAN

Wellcome Research Unit, Christian Medical College Hospital, Vellore 632 004 (India)

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An acid lipase (EC 3.1.1.3) from human gastric juice was purified by using poly(ethylene glycol)-6000 precipitation, ethanol fractionation and Sephadex G-75 gel filtration. A molecular weight of 44000 was obtained by SDS-polyacrylamide gel electrophoresis. pH-dependent aggregation was observed and by using Sephadex G-200 gel filtration, a molecular weight of 90000 was obtained at pH 6.0 and 45000 at pH 3.0, for the purified enzyme. A pH optimum of 5.3 was obtained using triolein as substrate. The apparent $K_m$ for tributyrin and triolein was found to be 21 and 73 µmol, respectively. Diacylglycerol and free fatty acids were the major hydrolytic end products of this enzyme. Studies on the positional specificity of the enzyme showed that the preferred site of hydrolysis was sn-3 and sn-1, although a good percentage of the sn-2 position was also hydrolysed. Conjugated bile salts inhibited the enzyme when triolein was used as substrate, whereas they activated it when tributyrin was used. Some of the properties of the purified human gastric juice acid lipase resembles those of rat and human lingual lipase.

Introduction

The presence of lipolytic activity in the gastric aspirates of human adults [1–3] and premature infants [4] has been reported. Evidence has accumulated for the existence of lipolytic activity in lingual serous (Von Ebner) glands of man [5] and other species [6]. Recently, Levy et al. [7] reported the presence of three different lipolytic enzymes of gastric, lingual and pancreatic origin in suckling rats. Gastric digestion of lipids leads to the hydrolysis of 10–30% of the ingested fat to partial glycerides and free fatty acids [2,3,8–11]. This facilitates the emulsification of triacylglycerols in the stomach [12]. Recent animal experiments have shown that the diversion of oral secretion from the stomach leads to a decrease not only in the intragastric lipolysis but also in the intestine [13]. 50–60% of ingested dietary triacylglycerols are absorbed in children with congenital absence of pancreatic lipase [10,14]. Hamosh et al. [6] partially purified the rat lingual lipase and studied some of the properties. Apart from this, there are no reports available on the purification of lipase either from gastric aspirates or from lingual glands. The present report describes the purification method and some properties of the acid lipase from human gastric juice.

Materials and Methods

Tributyrin was used as substrate for the enzyme during the purification process and the assay procedure was similar to that of Fredrikzon and Hernell [15] with slight modification. 1 ml of tributyrin was emulsified in 30 ml of 0.12 M citrate/phosphate buffer, pH 5.3, containing 0.15 M NaCl (buffer I) and 2.5 mM taurocholate. 2.5 ml of the above suspension was used for each assay. The incubation was at 37°C for 2–5 min in
a shaking water bath, the reaction was terminated and the butyric acid released was measured [16]. Triolein was used as substrate for studying the properties of the purified enzyme. 886 mg of triolein was emulsified in 10 ml of buffer I containing 3.6% bovine serum albumin and 1% gum arabic (buffer II). 2.5 ml of emulsified suspension was incubated with 25–75 µg of purified enzyme in a total volume of 3 ml at 37°C for 15–30 min and, after termination of the reaction, the fatty acids released were quantitated [17]. Under the conditions of assay the purified enzyme activity was linear with time up to 2 min when tributyrin was the substrate in the presence of 5 mM taurocholate and up to 30 min when triolein was used as substrate. We have observed that tributyrin hydrolysis was strongly inhibited when gum arabic was used as emulsifier.

Glyceroltri[1-14C]oleate (Radiochemical Centre, U.K.) was used for measuring the hydrolytic end-products. 89 mg of unlabelled triolein plus 1.4 µCi of glyceroltri[1-14C]oleate were emulsified in 10 ml of buffer II. 2.5 ml of this suspension was incubated with 25 µg of purified enzyme protein for various time intervals. The lipids were extracted into hexane by a slight modification [18] of the method of Dole and Meinertz [19] and then the various labelled lipolytic products were quantitated [9]. To study the positional specificity, 250 mg of glyceryl-1-palmitate-2-oleate-3-stearate (Calbiochem, U.S.A.) were emulsified in 3 ml of buffer II and in 0.12 M sodium phosphate buffer, pH 7.0, containing 0.15 M NaCl, 3.6% bovine serum albumin and 1% gum arabic and the reaction was carried out as described previously for 10 and 30 min. Free fatty acids were extracted [19], methylated [20] and quantitated on a 5% EGSS-X on a gas-chrom Q 80–100 mesh (2 m x 4 mm internal diameter) glass column using Pye-104 Gas Chromatograph.

Protein was determined by the method of Lowry et al. [21]. Polyacrylamide gel electrophoresis was carried out as described by Davis [22] at pH 9.5 (50 mM glycine/NaOH) and pH 4.0. The molecular weight of the purified enzyme was determined by SDS-polyacrylamide gel electrophoresis [23] and by Sephadex G-200 gel filtration at pH 6.0 and 3.0 [24].

After an overnight fast, gastric juice was collected [1] for a 1 h period, following which a 2.2 mg histamine intramuscular injection was given and the sample was collected over ice similarly for 1 h. Lipase activity was measured in fasting and histamine-stimulated gastric juice on 15 individual subjects. It was observed that after histamine stimulation the lipase specific activity was increased from 0.84–2.67-fold (mean, 1.618; S.D., 0.55) compared to fasting gastric juice.

All the purification steps were carried out at 4°C unless otherwise specified. Clear, colourless fasting and post-histamine juice was centrifuged at 12000 × g for 15 min and the supernatant pH was measured. The pH was adjusted to 3.0–3.3 with 5 N NaOH and the precipitate formed was removed by centrifugation at 12000 × g for 30 min. The pH of the supernatant containing the enzyme activity was adjusted to 6.0. To 100 ml of gastric juice (pH 6.0), a 10% solution (v/v) of Triton X-100 was added to a final concentration of 0.5% and stirred for 30 min. To this, a 50% solution of poly(ethylene glycol)-6000 in 0.01 M sodium phosphate buffer, pH 6.0, was added to a final concentration of 15% and stirred for 30 min. This was centrifuged at 12000 × g for 45 min and the precipitate was dissolved in 8–10 ml of 0.05 M citrate buffer, pH 3.0, containing 0.15 M NaCl (buffer A).
It was observed that inclusion of sodium chloride in the buffer was essential for the solubility and stability of the enzyme. To this, an equal volume of 50% ice-cold ethanol was added slowly with mixing, stirred for a further 10 min and centrifuged at 12000×g for 30 min. The supernatant containing enzyme activity was dialysed against buffer A and concentrated to 2–3 ml by lyophilization and subjected to Sephadex G-75 gel filtration. The column (65×1.5 cm) was equilibrated with buffer A and 3-ml fractions were collected. The elution profile of the enzyme is shown in Fig. 1A. The active fractions were pooled and used for further studies.

Results and Discussion

Table I summarises the purification steps and recovery of the enzyme. The enzyme was purified 15–25-fold depending on the initial protein concentration of the gastric juice with an overall recovery of 40–60%.

When subjected to polyacrylamide gel electrophoresis at pH 4.0 with 4% and 7.5% gel, the enzyme did not enter the gel. On the other hand, electrophoresis at pH 9.5, enzyme entered the gel and gave three diffusible protein bands in the same region extending to a length of 8 mM. On SDS-polyacrylamide gel electrophoresis the enzyme appeared as a single protein band (Fig. 1B) corresponding to Mr, 44000. The molecular weight of the enzyme was determined using Sephadex G-200 gel filtration at pH 6.0 and 3.0. The column (75–1.7 cm) was first equilibrated with 0.05 M sodium phosphate buffer, pH 6.0, containing 0.15 M NaCl (buffer B) and calibrated with known molecular weight markers. Original gastric juice at pH 6.0 gave Mr, 50000, whereas the purified enzyme when dialysed against buffer B, at pH 6.0, and passed through the same column gave Mr, 90000. The same column was equilibrated with buffer A (pH 3.0), and enzyme at pH 3.0 gave Mr, 45000. The Mr at pH 3.0 is in close agreement with that obtained by SDS-polyacrylamide gel electrophoresis. The appearance of three diffusible bands at pH 9.5 in polyacrylamide gel electrophoresis may be due to the pH-dependent aggregation of the enzyme. A Mr range of 44000–50000 was reported for the lipase in human gastric juice [1] and for the rat lingual lipase [6]. Our data shows that the purified enzyme at pH 6.0 aggregates to a dimer. However, the enzyme in the original gastric juice at pH 6.0 showed that it exists as a monomer, suggesting that factors other than the pH change may influence the aggregation of the enzyme.

The purified enzyme was used for studying various properties. The pH optimum of the enzyme in the presence of taurocholate with tributyrin as substrate was between 4 and 6 and with triolein was between 4.5 and 6.0 (Fig. 2). The pH optimum was also studied in the absence of taurocholate and with 1% gum arabic as emulsifier for the triolein substrate and it was found to be 5.3. This is similar to the pH optimum reported for the rat [6] and human lingual lipase [5] and different from the lipase of rat gastric mucosa [7].

Kinetic studies were carried out with tributyrin and triolein as substrates in the presence of 5 mM taurocholate at pH 5.3. The apparent $K_m$ for tributyrin and triolein was found to be 21 and 73 μmol, respectively, suggesting that short-chain tri-

**TABLE I**

**PURIFICATION OF LIPASE FROM HUMAN GASTRIC JUICE**

1 unit of enzyme activity corresponds to the liberation of 1 μmol of fatty acid per min under the conditions of assay.

<table>
<thead>
<tr>
<th>Step</th>
<th>Total activity (units)</th>
<th>Protein (mg)</th>
<th>Specific activity (units/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric juice at pH 6.0</td>
<td>8800.0 (100%)</td>
<td>425.00</td>
<td>20.70</td>
</tr>
<tr>
<td>0.5% Triton X-100 and 15% poly(ethylene glycol)-6000 precipitation</td>
<td>5000.0 (56.8%)</td>
<td>30.80</td>
<td>162.33</td>
</tr>
<tr>
<td>23% Ethanol fractionation</td>
<td>4543.0 (51.6%)</td>
<td>14.50</td>
<td>313.31</td>
</tr>
<tr>
<td>Sephadex G-75 gel filtration</td>
<td>4403.5 (50%)</td>
<td>9.25</td>
<td>476.05</td>
</tr>
</tbody>
</table>
Fig. 2. Effect of pH on the activity of purified lipase from human gastric juice. A, Tributyrin emulsified with 5 mM taurocholate as substrate. B, ○, triolein emulsified with 1% gum arabic and 3.6% bovine serum albumin; Δ, triolein emulsified with 5 mM taurocholate and 3.6% bovine serum albumin as substrate. The buffers were glycine-HCl for pH 2.5–3.5, citrate/phosphate buffer for pH 4.0–5.8, and phosphate buffer for pH 6.0–8.0. All other assay conditions are described in Materials and Methods.

Acylglycerols are hydrolysed faster than long-chain triacylglycerols.

To characterize the end-products of triacylglycerol hydrolysis, lipolysis was carried out using glyceroltri[1-14C]oleate. Fig. 3 shows the percentage of 1-14C present in various products after incubating the enzyme at various time intervals. More than 50% of triacylglycerol was hydrolyzed with 60 min. The major end-products were found to be free fatty acids and diacylglycerol. The 1-14C present in free fatty acid and diacylglycerol fractions was found to be more or less in equal proportion. This suggests that this acid lipase is capable of hydrolysing triacylglycerol completely to free fatty acids and glycerol, which is similar to reports on rat lingual lipase [6]. The positional specificity using glyceryl-1-palmitate-2-oleate-3-stearate showed that the enzyme released 25% of fatty acid from the sn-2 position of triacylglycerol (Table II). However, the most preferable position to this enzyme was sn-3 and sn-1. Thus, in positional specific cleavage, this enzyme resembles rat lingual lipase [7].

Fig. 4 shows the influence of different bile salts on lipolytic activity with tributyrin and triolein as substrates. Of the bile salts tested with tributyrin as substrate, except glycochenodeoxycholate, all others were found to activate the enzyme at various concentrations ranging from 2.5 to 10 mM. When triolein was used as substrate, none of the bile salts activated the enzyme but they were inhibitory. This is similar to the substrate-dependent inhibitory behaviour of bile salts on pancreatic lipase [25,26]. Our results on the effect of bile salts are contrary to the findings of Levy et al. [7] with rat lingual lipase, but concur with the observations on the lipolytic activity in the gastric aspirates of premature infants [4].

Phospholipase activity of the enzymes was tested at pH 5.3 and 7.0 [27] using phosphatidylcholine as substrate, and it was observed that the enzyme did not hydrolyse the above substrate. Thermal stability of this enzyme was studied at different temperatures. The enzyme lost 10 and 45% of initial activity at 42 and 52°C, respectively, after preincubating for 20 min, whereas at 62°C 100% inactivation was observed. This observation closely resembles that on rat lingual lipase [6].

Histamine and pentagastrin were shown to stimulate the lipolytic activity in the gastric mucosal slices of suckling rats [7]. In the 15 subjects from whom gastric juice was collected after histamine stimulation, we observed that the increase in lipase activity was not appreciably high. It is to be mentioned here that during gastric juice collection, swallowing of salivary secretion were not prevented. However, the enzyme from post-histamine gastric juice behaved very similar to fasting gastric juice in purification process and in all properties studied.

In conclusion, the purified lipase from human gastric juice resembled rat lingual lipase in its molecular weight, pH optimum, lipolytic end-
TABLE II
LIPOLYSIS OF GLYCERYL-1-PALMITATE-2-OLEATE-3-STEARATE
A, 10 min incubation; B, 30 min incubation. Other details are as described in the text.

<table>
<thead>
<tr>
<th>pH</th>
<th>Free fatty acids (%) in lipolysis products</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>C16:0</td>
</tr>
<tr>
<td>5.3</td>
<td>33.7</td>
</tr>
<tr>
<td>7.0</td>
<td>36.0</td>
</tr>
</tbody>
</table>

Fig. 4. Effect of different bile salts on the lipolytic activity of lipase. A, Tributyrin; B, triolein as substrate. Tributyrin was sonicated with 0.12 M citrate/phosphate buffer containing 0.15 M NaCl at pH 5.3 for control assay. Triolein was emulsified with 1% gum arabic and 3.6% bovine serum albumin at pH 5.3 for control assay. To test the effect of various bile salts, different concentrations of bile salts were emulsified with control assay. Values are percentages of lipolytic activity without bile salts. TC, taurocholate; TCDC, taurochenodeoxycholate; TDC, taurodeoxycholate; GC, glycocholate; GDC, glyccodeoxycholate; GCDC, glycochenodeoxycholate.

products, positional specificity and thermal stability, and differed from rat gastric lipase [7] on the above properties, suggesting that the origin of this acid lipase in gastric juice may be the lingual glands.

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References

10 Muller, D.P.R., McCollum, J.P.K., Trompeter, R.S. and Harries, J.T. (1975) Gut 16, 838