Prolonged Hyperlipasemia Attributable to a Novel Type of Macrolipase

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Background: We present the case of an 80-year-old woman who was admitted to hospital with an intermittent volvulus of the right colon. A total colectomy was performed. Initially, serum amylase and lipase increased concordantly, but after a few weeks amylase normalized (85 U/L), whereas lipase increased to 3764 U/L. This discrepancy and persistence of hyperlipasemia suggested a macromolecular form of lipase.

Methods: The nature of the macromolecular complex was studied using high-pressure liquid gel-permeation chromatography, affinity chromatography, (immuno)electrophoresis, and immunodiffusion.

Results: Gel-permeation chromatography revealed a macrolipase, with a molecular mass >900 kDa, that contributed up to 56% of total serum lipase activity. Butanol extraction of the specimen did not alter the elution profile. The thermostabilities of pancreatic lipase and the macroform were similar, whereas activation energy (E_a) was lower in the macromolecular lipase (28 ± 4 kJ·mol⁻¹·K⁻¹ vs 48 ± 7 kJ·mol⁻¹·K⁻¹ (P = 0.02). Agarose electrophoresis showed a broad band of lipase activity at the application site. Protein A-Sepharose affinity gel chromatography excluded IgG-linked lipase. Agarose electrophoresis and immunofixation excluded linkage to other immunoglobulins. Radial immunodiffusion did not show lipase activity in the immunoglobulin precipitation bands. Radial immunodiffusion with α2-macroglobulin (α2-MG) antibodies showed a diffuse spot of lipase activity within the precipitation band, suggesting a macromolecular association between lipase and α2-MG. Affinity gel chromatography against α2-MG showed lipase activity in the α2-MG-bound fractions.

Conclusion: This is the first report of a macrolipase in which an association between α2-MG and lipase is described.

Determinations of serum lipase (triacylglycerol acylhydrolase; EC 3.1.1.3) and amylase (1,4-α-D-glucan glucanohydrolase; EC 3.2.1.1) activities are important in the diagnosis of acute pancreatitis (1, 2). Macroenzymes (3–5) are enzymes of high molecular mass that are formed in serum by self-polymerization or by association with other proteins. Because of their high molecular mass, they escape normal glomerular filtration and accumulate in plasma. The nature of these macroenzymes in the majority of cases is an association with an immunoglobulin (IgG or IgA). Most of the serum enzymes routinely measured in the clinical laboratory have been described in lipid aggregates or exhibiting immunoglobulin macroforms (6–11).

Although the clinical importance of macroenzymes is limited, the presence of a macroenzyme can be misleading in the interpretation of routine laboratory results (12), which can lead to a false conclusion of disease and to further expensive and dangerous investigations, or it can mask active disease (13). The prevalence of macroenzymes increases with age, but they are seldom encountered in healthy individuals. Therefore, their presence should alert the clinician to possible underlying conditions.

Only a few reports exist on the macromolecular forms of lipase; all these macrolipases are immunoglobulin-associated. Stein et al. (14) reported an IgG-bound lipase in a patient with a non-Hodgkin lymphoma. Zaman et al. (15) described simultaneous macrolipasemia and macroamylasemia in association with poly-IgA. Two Japanese reports described an IgG- and an IgA-bound macrolipase (16, 17). Other reports concerning undetermined hyperlipasemia could not demonstrate an association (18–20).

In this report, we aim to demonstrate the presence of a macrolipase and to describe its biochemical and biophysical characteristics.

References:
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Case Report

An 80-year-old woman was admitted to the hospital with chronic constipation and progressive abdominal distension. The medical history consisted of a cholecystectomy 20 years ago and no evidence of alcohol abuse. Table 1 summarizes the initial laboratory values. The profiles for serum lipase and amylase are illustrated in Fig. 1. Radiological contrast examination of the abdomen revealed an elongated (dolicho-) colon, diverticulosis of the sigmoid, and an intermittent volvulus of the right colon. A total colectomy with ileo-rectal anastomosis was performed. Before surgery, she received cefazolin, metronidazole, and diazepam. Anesthesia was initiated with thiopental, cisatracurium besylate, and fentanyl and was maintained with desflurane, N₂O, and O₂. No signs of malignancy or inflammatory bowel disease were observed. After surgery, there was a prolonged ileus, which resolved spontaneously.

When oral nutrition was reintroduced, the patient started vomiting. A laparotomy revealed that the anastomosis was intact, but small bowel strangulation was corrected by adhesiolysis. The pancreas was indurated upon palpation, and there were signs of peritoneal inflammation.

No signs of pancreatitis were observed on a computed tomography scan. Endoscopic retrograde cholangiopancreatography showed minimal dilatation of the choledochus and Wirsung duct. In addition, an endoscopic sphincterotomy was performed.

Two months later, the patient relapsed because of partial obstruction of the small bowel caused by adhesions on the anterior abdominal wall. After adhesiolysis, the ileum regained its normal appearance. During this prolonged episode, the patient was administered several types of medication, including tramadol, propacetamol, enoxaparin, digoxin, amiroidane, furosemide, atenolol, cefuroxime, metilimicin, cefazolin, pefloxacin, metronidazole, ondansetron, alziprider, pantoprazole, haloperidol, and diazepam.

After 250 days of follow-up, the patient was in good health and had inflammatory marker concentrations and serum lipase and amylase activities within reference values.

Materials and Methods

Specimens and Specimen Treatment

Serum specimens were either analyzed on the day they were obtained or were stored at −20 °C until analysis. In all other experiments described here, we used the specimen collected on day 21. The lipase activity of this specimen was 1207 U/L (Vitros). Before high-pressure liquid gel-permeation chromatography (HPLGPC), the specimen was treated with n-butanol (300 mL/L) to dissociate lipid-or membrane-bound enzymes.

Enzyme Assays

We determined serum lipase activity at 37 °C using three different methods: (a) turbidimetric (triolein ultraviolet method; cat. no. 1661787; Roche Diagnostics; reference values, 0–190 U/L); (b) colorimetric (6-methylresorufin; cat. no. 182176; Roche Diagnostics; reference values, 0–60 U/L); and (c) dry-film reagent slides (Vitros LIPA slides; cat. no. 8374191; Johnson & Johnson Clinical Diagnostics; reference values, 23–300 U/L). Other serum enzymes were measured at the same time: alkaline phosphatase (EC 3.1.3.1), amylase, γ-glutamyltransferase (EC 2.3.2.2), lactate dehydrogenase (EC 1.1.1.27), aspartate aminotransferase (EC 2.6.1.1), and alanine aminotransferase (EC 2.6.1.2).

All assays were performed according to the manufacturers’ instructions at 37 °C on a Hitachi 747 analyzer (Roche Diagnostics), except for the dry-film lipase determinations, which were performed on Vitros LIPA slides.

Table 1. Initial routine laboratory results.

<table>
<thead>
<tr>
<th>Patient’s results</th>
<th>Reference values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood sedimentation rate at 60 min, mm 41</td>
<td>0–30</td>
</tr>
<tr>
<td>White blood cell count, 10⁹/µL 13.1</td>
<td>4.5–12</td>
</tr>
<tr>
<td>AST,a U/L 182</td>
<td>0–31</td>
</tr>
<tr>
<td>ALT, U/L 239</td>
<td>7–31</td>
</tr>
<tr>
<td>ALP, U/L 120</td>
<td>42–98</td>
</tr>
<tr>
<td>γGT, U/L 254</td>
<td>7–32</td>
</tr>
<tr>
<td>LD, U/L 989</td>
<td>231–462</td>
</tr>
<tr>
<td>Amylase, U/L 57</td>
<td>30–110</td>
</tr>
<tr>
<td>Lipase, U/L 575</td>
<td>23–300</td>
</tr>
</tbody>
</table>

a AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; γGT, γ-glutamyltransferase; LD, lactate dehydrogenase.

Fig. 1. Serum lipase (○) and amylase (●) values vs time as measured by the Vitros apparatus. Reference values: lipase, 23–300 U/L; amylase, 30–110 U/L. (-- -- --), lipase upper reference limit (300 U/L); (· · · · · · · · · ·), amylase upper reference limit (110 U/L).
NEPHELOMETRY
Postcolumn α2-macroglobulin (α2-MG) concentrations were determined nephelometrically on a BNA II nephelometer (N antisera to human α2-MG; Dade Behring).

HPLGPC
A 50-μL serum specimen was applied to a 0.9 × 30 cm silica gel column (300 SW Protein Pak; Waters) with a phosphate buffer (0.1 mol/L, pH 7.4) as the mobile phase; the sample was eluted at 0.8 mL/min. A fraction collector was used to collect 13 0.8-mL fractions. IgM (900 kDa), IgG (150 kDa), and albumin (68 kDa) were used as molecular mass references. Postcolumn lipase activities were determined by the turbidimetric (triolein) method. This procedure was applied to both untreated serum specimens and butanol-extracted specimens.

CATALYTIC ACTIVITY
The activation energies (Ea) of both the macroform and unbound pancreatic lipase were calculated using the Arrhenius relationship (21). We determined lipase activity turbidimetrically (triolein) at 25 and 37 °C.

The thermostability of both the macroform and normal pancreatic lipase was tested by continuously monitoring lipase activity turbidimetrically during heating of the serum specimen up to 55 °C (21).

AFFINITY GEL CHROMATOGRAPHY
Protein A-Sepharose column. A 500-μL serum specimen was adsorbed on a (5 × 0.5 cm) column packed with protein A-Sepharose CL-4B (Pharmacia). Non-IgG constituents were eluted with 0.5-mL fractions of 50 mmol/L Tris buffer, pH 8.0. IgG was eluted from the column with 0.5-mL fractions of 0.5 mol/L acetic acid. Fractions were adjusted to physiological pH by dialysis for 3 h against a 9 g/L NaCl solution before colorimetric determination of lipase activity. Lipase activities in the α2-MG fractions were measured colorimetrically (6-methylresorufin) after incubation for 20 min at 37 °C.

ELECTROPHORESIS
Electrophoresis and immunoelectrophoresis were carried out in agarose gels using a barbital buffer (pH 8.6; ionic strength = 0.05) for 30 min at 100 V (Paragon immunofixation electrophoresis method; Beckman).

Lipase activity was visualized using a sandwich technique. The lipase-reagent layer was prepared by dissolving the colorimetric substrate (6-methylresorufin) in 2% agarose gels (50%, by volume). Co-lipase (1 mg/L) and sodium deoxycholate (1.6 mmol/L) were added to these substrate agarose strips. The electrophoretic gels were overlaid with these agarose strips, allowing visualization of the lipase activity.

In addition, agarose electrophoresis was performed, using “special purpose electrophoresis film” (cat. no. 470104; Ciba-Corning) and MOPSO buffer (cat. no. 470046; Ciba-Corning) as described previously (2). Lipase was visualized using lipase dry slides (Vitros). The procedure was carried out as recommended for creatine kinase isoenzymes by the manufacturer.

Both techniques were performed on the macrolipase specimen and a control specimen from a healthy individual.

IMMUNOLOGICAL METHODS
Radial immunodiffusion disks were prepared by dissolving 30 mL/L of a specific antiserum against human immunoglobulins (IgG, IgM, and IgA) and α2-MG in 2% agarose. These disks were stained for lipase activity, using fractions of 0.5 mol/L acetic acid. Fractions were adjusted to physiological pH by dialysis for 3 h against a 9 g/L NaCl solution before colorimetric determination of lipase activity.
the agarose-lipase substrate (colorimetric strips with 6-methylresorufin), as described above, as a template.

Results

The presence of a macrolipase was suspected because serum lipase activities were persistently increased during the initial follow-up period (Fig. 1; Vitros dry LIPA slides), as measured by three different methodologies. The values obtained by the three methods for the patient’s sample on day 21 (Fig. 1) were as follows: turbidimetric (triolein), 490 U/L (reference values, 0–190 U/L); colorimetric (6-methylresorufin), 254 U/L (0–60 U/L); Vitros LIPA slide, 1207 U/L (23–300 U/L); amylase activity, however, returned to reference values.

HPLGPC

A macrolipase was confirmed by HPLGPC. Fig. 2 shows the gel-permeation chromatogram for lipase activity (turbidimetric method with triolein), revealing a macromolecular form of lipase that eluted in the void fraction, with an estimated molecular mass of >900 kDa. Normal pancreatic lipase has a molecular mass of 50 kDa (23). The macroform accounted for 56% of the total serum lipase activity. The elution pattern of α2-MG matched the pattern for lipase. Butanol extraction of the specimen retained the general elution pattern and excluded lipid- or membrane-bound lipase. None of the screened serum enzymes (alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, amylase, and γ-glutamyltransferase) were included in this high-molecular mass complex.

Thermostability and activation energy

The thermostability of both forms was comparable. The macroform was inactivated at 51.3 °C vs 51.7 °C for normal-sized lipase. The \( E_a \) was lower in the macromolecular lipase: 2864 vs 3867 kJ mol\(^{-1}\) K\(^{-1}\) (\( P = 0.02 \)) for the unbound lipase.

Affinity gel chromatography

Protein A-Sepharose column. Protein A-Sepharose affinity gel chromatography did not show lipase activity in the IgG-bound fractions (Fig. 3). The e-\( \alpha_2 \)-MG concentrations are also plotted in Fig. 3. Lipase activity was observed in the e-\( \alpha_2 \)-MG-bound fractions.

Anti-\( e-\alpha_2 \)-MG-Sepharose column. The e-\( \alpha_2 \)-MG affinity-chromatography acid elution pattern is shown in Fig. 4. e-\( \alpha_2 \)-MG concentrations are also plotted in Fig. 4. Lipase activity was observed in the e-\( \alpha_2 \)-MG-bound fractions.

Electrophoresis and immunological methods

Agarose electrophoresis (Fig. 5) showed a broad band of lipase activity at the application site, extending toward the cathode. The control specimen showed one band of
lipase activity on the 6-methylresorufin-agarose strips. Dry slide lipase visualization revealed the characteristic pattern described previously (2).

Immunofixation failed to show any immunoglobulin-linked (IgG, IgA, or IgM) lipase complex. In addition, radial immunodiffusion did not show any lipase activity within the immunoglobulin precipitation bands. Radial immunodiffusion with anti-human \( \alpha_2 \)-MG antibodies showed a diffuse spot within the precipitation band. (Note: The results for the immunological tests are not shown.)

**Discussion**

To our knowledge, this is the first report in which a complex between pancreatic lipase and \( \alpha_2 \)-MG is described. \( \alpha_2 \)-MG is a carrier of various proteolytic and nonproteolytic proteins, such as cytokines, growth factors, hormones, and several peptides, and it binds serine-, thiol-, carboxyl-, and metallo-proteases (24). \( \alpha_2 \)-MG inhibits the activity of the bound protease in the circulation, protecting the tissues from autodigestion. The binding of a protease to \( \alpha_2 \)-MG causes a conformational change in \( \alpha_2 \)-MG. Nevertheless, the complex retains some residual proteolytic activity. When \( \alpha_2 \)-MG-trypsin complexes are exposed to acid conditions (pH 3.7), the complex dissociates and free trypsin is released, expressing proteolytic activity (24). The binding of a nonproteolytic protein to \( \alpha_2 \)-MG is less characterized and causes no obvious conformational change.

Both free and \( \alpha_2 \)-MG-bound lipase exhibited catalytic lipase activity. Affinity chromatography against \( \alpha_2 \)-MG revealed two peaks with lipase activity (Fig. 4), which can be explained by the gel-permeation effect of Sepharose 4B. However, the \( \alpha_2 \)-MG concentrations did not match the observed lipase activities. The maximal lipase activity was in fraction 6, and the highest \( \alpha_2 \)-MG concentration was in fraction 3, indicating that the acid elution caused dissociation of the \( \alpha_2 \)-MG-lipase complex, as described for trypsin (24).

In the propositus, lipase had undergone conformational change, explaining the altered \( E_a \).

We tried to exclude as much nonpancreatic lipase (intestinal lipase, cholesterol esterase, and other esterases) as possible by measuring lipase activities with substrates specific for the pancreatic form (triolein ultraviolet method, and 6-methylresorufin). The Vitros lipase substrate (formerly known as the Kodak Ektachem system) is particularly prone to cross-reaction with nonpancreatic lipase. The Vitros system uses 1-oleoyl-1,3-diacyl glycerc, which has one long-chain (oleic acid) and two short-chain (acetic acid) esters. Nonpancreatic lipases and esterases have been shown to hydrolyze this substrate more effectively than pancreatic lipase (25, 26).

Several studies in the literature have reported persistent hyperlipasemia (19, 20, 27, 28), but were not able to demonstrate any macrolipase or could not identify the association (18). We speculate that, except for hyperlipasemia attributable to cross-reaction with other esterases, as measured on the Vitros apparatus, this hyperlipasemia could be the result of an association with \( \alpha_2 \)-MG.

In contrast to immunoglobulin-bound macroenzymes, the hyperlipasemia was present for only 3 months. The transient nature of the prolonged hyperlipasemia suggests that the association between lipase and \( \alpha_2 \)-MG originated during the acute phase and persisted for several months after the pancreatitis because of the diminished clearance of the complex. Another explanation could be the weak binding forces of this association compared with immunoglobulin-bound macroenzymes. However, butanol extraction of the specimen did not break up the complex, thus illustrating its relative stability. The prolonged hyperlipasemia produced by this association can cause confusion in the diagnosis or follow-up of pancreatitis, and clinicians should consider the presence of macroenzymes when amylase and lipase activities are discrepant.

**References**