Simultaneous Macroamylasemia and Macrolipasemia

Zahur Zaman,1,4 Angeline Van Orshoven,1 Godelieve Mariën,2 Johan Fevery,3 and Norbert Blanckaert1

The first case of the simultaneous presence of macroamylasemia and macrolipasemia in a patient with gluten enteropathy (celiac disease) is described. Both macroenzymes were formed by association of polyclonal IgA with amylase and lipase. Both macroenzymes had molecular masses >300 kDa.

Indexing Terms: celiac disease/enzyme–immunoglobulin complex/es/macroenzymes

Biochemically, forms of serum enzymes with greater than normal molecular mass (macroenzymes) are most commonly formed by the complexing of a normal (iso)enzyme with an immunoglobulin (1). They may also occur as oligomeric complexes of isoenzymes, in association with a membrane fragment or with lipoprotein-X (1). Because they are cleared much more slowly than the usual enzymes, macroenzymes accumulate in the plasma and thus cause an increase in the activity of the corresponding enzyme in blood samples (2). The existence of macroenzymes is usually considered in situations of atypical clinical features associated with abnormally high concentrations of enzyme.

Clinically, macroenzymes are important for two reasons: They cause confusion in the interpretation of the serum enzyme results and may occasionally be associated with a pathology (2, 3). We describe a patient with celiac disease (also called gluten enteropathy) who has had persistently above-normal serum concentrations of amylase and lipase activities. We found that the abnormally high activities were due to the presence of polyclonal IgA complexed with amylase and lipase. To our knowledge, this is the first description of the simultaneous presence of macroamylase and macrolipase in the same patient.

Materials and Methods

Procedures

Enzyme activities. Total amylase activities were determined on BM/Hitachi 911 and BM/Hitachi 747 automated analyzers with a kit from BioMérieux (Marcy-L’Etoile, France). Total lipase activities were measured with the Ektachem 700 XR (Eastman Kodak, Rochester, NY).

Electrophoresis. Amylase samples were electrophoresed on agarose gel in Tris-borate buffer, pH 6.8, and stained for isoenzymes as described in the Beckman (Beckman Instruments, Fullerton, CA) isoamyl kit developed by Analis (Namur, Belgium). For lipase, we followed the Beckman procedure for electrophoresis of creatine kinase isoenzymes except that the MOPS buffer was adjusted to pH 7.8 with NaOH. Lipase isoenzymes were visible after being overlaid with Kodak lipase reagent strips (4). Immunofixation of 10-fold-diluted serum was performed on agarose gel in barbital buffer, pH 8.6, with Beckman’s Paragon Masterplan immunofixation electrophoresis method. Immunoelectrophoresis was carried out on agarose gel in barbital buffer, pH 8.6, according to the procedure from Kallestad Labs. (Austin, TX). Kappa and lambda light chains were determined with a Behring nephelometric analyzer (Behringwerke, Marburg, Germany) and Behringwerke antisera and calibrators.

Gel exclusion chromatography. Macroamylase and macrolipase were also separated from the smaller molecular forms by gel exclusion chromatography on a 1 × 30 cm column of Superose 12 HR 10/30 (Phannacia, Uppsala, Sweden), mean particle size 10 μm, developed in 50 mmol/L phosphate buffer, pH 7.4, at an elution rate of 0.45 mL/min.

Case Report

A 56-year-old man had presented in 1972 with persistent diarrhea. Biochemically he showed generalized malabsorption with steatorrhea. His serum IgA, IgM, and amylase were increased; his urine amylase was low normal. Biopsies of the small intestine showed incomplete villus atrophy. The patient was diagnosed as having gluten enteropathy (celiac disease) and therefore was started on a gluten-free diet. The persistently high serum amylase was subsequently confirmed electrophoretically to be due to the presence of macroamylase.

The patient has been closely followed at this institution. He has had many flare-ups, which have always been associated with nonadherence to a gluten-free diet. At his most recent admission, attributable to a weight loss of 16 kg over 6 months, some of the relevant laboratory test results (and reference ranges) for plasma analytes were: alkaline phosphatase 251 U/L (90–260), aspartate aminotransferase 44 U/L (<40), alanine aminotransferase 35 U/L (<40), γ-glutamyltransferase 16 U/L (11–50), lactate dehydrogenase 479 U/L (150–460), lipase 981 U/L (23–208), amylase 856 U/L (30–85), and IgA 17.4 g/L (0.71–4.17). Concentrations of plasma elec-
trolites, urea, creatinine, and bilirubin were normal. Urinary amylase was <2 U/L (<390 U/L) and urinary protein was 1.4 g/24 h (<150 mg/24 h). Fecal fat, measured when the patient was not taking oral exocrine pancreatic supplement (pancreatin), was 334 mmol/day (<18 mmol/day); during treatment with pancreatin supplement, it decreased to 186 mmol/day.

Results
Electrophoresis and Gel Filtration Chromatography

Figure 1 shows the electrophoretic patterns of lipase and amylase isoenzymes in blood samples from the patient described above, from another patient recovering from pancreatitis, and from a control subject. The plasma from our patient contained isoenzymes of both amylase and lipase, which were not present in the control subject. However, although a trace of normal lipase corresponding to fraction L1 (Fig. 1A, lane 6) was present in the patient being investigated, no normal fractions of amylase were detectable. Fig. 2 shows the elution profiles of lipase and amylase activities in these patients’ samples, individually applied to the Superose 12 HR 10/30 column. Nearly 95% of the amylase and lipase activities were contained in fractions eluting with the void volume; the remaining 5% of the activities eluted in fractions coincident with those from the patient recovering from pancreatitis (Fig. 2).

On the Superose column, the early-eluting lipase activities from the study subject were resolved into two peaks (Fig. 2A). Aliquots of the fractions corresponding to the peak activities (fractions 18 and 22) were electrophoresed and stained for protein and lipase activity. Both fractions produced broad single bands of lipase activity. Mixture of the two fractions also gave a single band (Fig. 1A, lane 7). Staining for proteins, however, revealed two protein bands in each fraction. The major band in each fraction had the electrophoretic mobility of an immunoglobulin. However, although the minor band in fraction 18 migrated to a position midway between α1 and α2, the minor band in fraction 22 was only marginally slower than the α1 band (Fig. 1A). Treating aliquots of fractions 18 and 22 with mercaptoethanol did not alter the mobilities of the enzyme or protein bands.

![Diagram of electrophoresis patterns](image)

**Fig. 1.** Diagrammatic representations of electrophoresis for lipase isoforms (A) and for isoamylase (B) of specimens from the patient thought to have macroamylase and macrolipase and from control subjects. *, application points on the gel. (A) Specimens: 1–4 were stained for protein and 5–7 for lipase activity. 1, normal subject; 2, study patient; 3, fraction 18 (see Fig. 2A); 4, mixture of fractions 18 and 22; 5, lipase from a patient recovering from pancreatitis (total lipase activity 4552 U/L); 6, lipase from study patient; 7, lipase from fractions 18 and 22. (B) Specimens: 1, human saliva; 2, a patient recovering from pancreatitis (total amylase activity 782 U/L); 3, study patient.

**Fig. 2.** Gel filtration chromatogram showing elution profiles of lipase activity (A) and amylase activity (B) in specimens from the study patient (■) and a patient recovering from pancreatitis (▲). Fraction volume 0.45 mL, elution rate 0.45 mL/min.

Immunological Studies and Enzyme Activities

Electrophoresis of our patient’s serum revealed a broad immunoglobulin zone anodal to the application point (Fig. 3). Immunofixation showed that the predominant immunoglobulin was IgA and that it contained both kappa and lambda light chains (Fig. 3A, lanes 5 and 6). Immunoelectrophoresis of the sample produced a thick IgA arc (Fig. 3B, lane 4C) and symmetrical thickening of the arcs for both kappa and lambda light chains (Fig. 3B, lane 6). Quantification of the light chains gave 13 and 5.2 g/L for kappa and lambda, respectively, with a kappa/lambda ratio of 2.5 (reference range 1.9–2.8).

During a period of ~9 months, we measured the serum amylase and lipase activities of our patient seven times. The means (and ranges) of these activities were 843 (645–1104) U/L for amylase and 1339 (729–2600) U/L for lipase. On all these occasions, urinary amylase activity was barely detectable (<2 U/L). In addition to IgA concentrations being higher than normal, we ob-
served that the total amylase and the total lipase activities fluctuated with changes in IgA and anti-gliadin IgA concentrations. For example, for IgA concentrations of 24 g/L, the amylase and lipase activities were 1104 and 1817 U/L, respectively; −6 months later, for IgA of 17 g/L, they were 645 and 759 U/L. On these two occasions the corresponding anti-gliadin IgA concentrations were 529 and 315 arb. units (1 arbitrary unit being defined as the amount of antibody for which the absorbance is 1% of that of a highly positive reference serum; reference range = <20 arb. units). This apparent correlation between the immunoglobulin concentrations and the total enzyme activities, and the fact that >90% of the total amylase and lipase activities were associated with IgA, suggested that increases in IgA concentration led to increased macroenzyme formation.

The patient’s serum was also examined for macrocreatinine kinase and macroalkaline phosphatase. Neither was found.

Discussion

Serum amylase or lipase or both are commonly measured to evaluate patients thought to have pancreatitis. The macromolecular forms of amylase and lipase are not filtered by the glomerulus and are slowly cleared from the plasma (2), leading to hyperamylasemia and hyperlipasemia. If a patient with unrecognized presence of macroamylase and macrolipase experiences an episode of acute abdomen and these enzymes are measured in his plasma, the activities of both enzymes would be above normal, and the patient would immediately be labeled as having pancreatitis. Such a misdiagnosis can lead to unnecessary, expensive, and sometimes invasive procedures. Therefore, the possible presence of macroenzymes should be entertained in differential diagnosis, particularly whenever the test results are discordant with the clinical picture.

In our patient, macroamylasemia had been detected electrophoretically nearly 20 years ago. Detection of his macrolipasemia had to wait, because good routine methods for lipase determinations have not been available until recently. This is reflected in the fact that, although macroamylasemia is not uncommon (1, 2), to our knowledge there have been only two previous reports of macrolipasemia (5, 6). The possibility that abnormal lipase was present arose for the same reason as for amylase—persistently above-normal lipase activity in the absence of any other signs of pancreatitis and fluctuations in the activities of both amylase and lipase mirroring the course of the patient’s gluten enteropathy, reflected in his concentrations of IgA and anti-gliadin IgA.

The abnormal forms of lipase and amylase were detected by electrophoresis on agarose gel (Fig. 1). Nearly 95% of the total lipase activity was associated with a band moving roughly midway between L1 and L2 fractions of the patient recovering from pancreatitis. The slight remaining activity was located in the fraction corresponding to L1 (Fig. 1A). That the abnormal fractions of amylase and lipase were macroenzymes was established by gel filtration chromatography on Sephacryl S-200, which has a fractionation range of 1–300 kDa. Nearly 95% of the applied activities of both amylase and lipase eluted in the void volume, corresponding to molecular masses >300 kDa. The remaining activities, coeluting with the normal enzyme fractions, corresponded to molecular masses of 35 kDa for lipase and 52 kDa for amylase, molecular masses that agree quite well with those reported for these enzymes (7, 8).

The presence of a smeared pattern for immunoglobulin on electrophoresis is not an evidence for polyclonality of the immunoglobulin in an Ig−enzyme complex, given that a monoclonal IgA plasma cell can give a similar picture (9). From the electrophoretic pattern alone, therefore, the immunoglobulin in our patient’s macroenzyme complexes could be mono- or polyclonal. However, the formation of a thick IgA arc and of symmetrically thick arcs for both kappa and lambda light chains on immunoelectrophoresis, the presence of kappa and lambda light chains in the smeared pattern on immunofixation, and a kappa/lambda ratio of 2.5 lead us to conclude that our patient had a polyclonal increase of IgA in his serum. Because macrolipase, despite eluting in the void volume, had resolved into two peaks and because most of the lipase was associated with polyclonal IgA, this raised the possibility that macrolipase might exist in association with IgA and (IgA)2. This

Fig. 3. (A) Electrophoresis and immunofixation of the serum from the study patient: lane 1, serum electrophoretic pattern; lanes 2–6, immunofixation for IgG, IgA, IgM, kappa light chain, and lambda light chain, respectively; (B) immunoelectrophoresis of a control serum (lanes 1, 3, 5, and 7) and serum from the study patient (lanes 2, 4, and 6) with antiserum to: A, whole human serum; B, human IgG; C, human IgA; D, human IgM; E, human kappa light chain; and F, human lambda light chain.
possibility was not substantiated, however, because treatment of the samples with mercaptoethanol failed to alter the chromatographic and electrophoretic behavior of the macrolipase fractions. This and the molecular mass >300 kDa indicate that the linkage between lipase and IgA is polymeric rather than one to one. The same is probably true for amylase. However, this does not exclude the association of the enzyme–IgA complex with other protein(s).

In conclusion, we have described a patient with persistently increased plasma concentrations of both amylase and lipase activities and established that this was due to the complexing of amylase and lipase isoenzymes with polyclonal IgA. To our knowledge, this is the first report of simultaneous occurrence of macroamylasemia and macrolipasemia.

We thank M. Zeegers, G. Gevers, and L. Sneyers for their technical assistance and B. Smeyers for the artistic work.

References