A Case of IgA-Binding-Type Macroamylasemia

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The serum amylase of an 83 year old man with no abdominal complaints, continuously high serum amylase activity and a remarkably low amylase clearance/creatinine clearance ratio was investigated and found to be IgA-binding type macroamylase. In electrophoresis of the serum amylase, a great majority of the phoretic band was in the globulin fraction. In Sephadex G-200 column gel chromatography, almost all amylase activity was found between the first and second protein peaks. When the patient's serum was acidified in vitro (pH 3.4), the amylase became normal in size and the change was reversible. The macroamylase was precipitated with anti-human IgA sheep serum. In enzymo-immunoelectrophoresis, amylase activity was found only in the IgA band. The urinary and salivary amylase in this patient was normal in size.

Key Words: Amylase, IgA-binding macroamylase, Column gel chromatography, Enzymo-immunoelectrophoresis.

Low urinary amylase activity and low amylase clearance/creatinine clearance ratio (Cam/Ccr) with continuously high serum amylase activity are sufficient basis for a clinical diagnosis of macroamylasemia. The binding substance which makes the amylase macromolecular is usually immunoglobulin, but there are cases in which some other unknown substances make this change.

Recently, the authors experienced one case of macroamylasemia and investigated the properties of his abnormal amylase.

CASE PRESENTION

The patient was an 83 year-old male. He had suffered from palpitation for 5 years. Although he had no other complaints, he had been found to have hyperamylasemia by his family doctor, and he consulted the Kinki University Hospital in May, 1977. He had been drinking two deciliters of sake (alcohol: 16%) every day for 20 years. He was a normally developed and well-nourished man (height: 161 cm, weight: 55 kg). His pulse was 72
per minute and irregular. His blood pressure was 110/72 mmHg. A systolic murmur was present in the fourth intercostal space at the left sternal line of the chest. The lungs showed no abnormality. The liver and spleen were not palpable and there was no swelling of the lymph nodes. The ECG showed atrial fibrillation, and only gastroptosis was seen in the upper GI series.

Laboratory data (Table 1):

Serum bilirubin was 2.1 mg/dl, serum amylase was as high as 3,552 IU, and the Cam/Ccr was as low as 0.09%. The total serum protein fractions, and IgA, IgG and IgM were all within the normal ranges.

MATERIALS AND METHODS

The specimens studied were serum, saliva and urine (Concentrated 25-fold with a PM 10 Amicon membrane) obtained from the patient. Sera obtained from a patient with gallstones (serum amylase: 620 IU, Cam/Ccr: 1.8%), and from two healthy persons who had normal serum amylase activity and normal Cam/Ccr were used as controls for gel filtration and electrophoresis, respectively. Amylase was measured by the modified method of Caraway (Wako Amylase Kit, expressed as “units”) or the blue starch polymer method (expressed as “IU”). Amylase isoyme fractionation was performed by electrophoresis on a cellulose acetate membrane stained with blue starch.

Measurement of amylase molecule size: Sephadex G-200 gel filtration (eluent: physiological saline, column: Φ 2.5 × 100 cm) was used. One milliliter of serum, 0.5 ml of saliva and 2.1 ml of concentrated urine of the patient and 2 ml of the control serum was applied to the gel. The protein content and amylase activity of the respective fractions were measured. To observe the normalization of the amylase size by the acidification of the macroamylase, 5.1 ml of glycine HCl (0.05 M, pH 3.4) was added to 1.7 ml of patient’s serum and

Fig. 1. Electrophoretic separation of serum amylase isoenzymes. Top and bottom levels show control sera from two normal volunteers and the middle two levels patient’s serum. The arrow (↑↓) shows the origin. The anode is to the left. The amylase of the control sera moved from the origin toward the cathode and the amylase of the patient’s serum moved mostly toward the anode.

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Fig. 2. Elution patterns of proteins and amylase from Sephadex G-200 (ø2.5×100 cm)

The solid lines show amylase activity and the broken lines the amount of protein.

(A) Control serum: amylase activity was found after the third protein peak.

(B) Patient's serum: amylase activity was high between the 1st and 2nd protein peaks.

(C) Patient's saliva and (D) concentrated patient's urine: The amylase activity for both C and D showed the same elution pattern as normal serum.

(E) Acidified patient's serum (pH 3.4): The molecular weight of the abnormal amylase was normalized.

after standing at room temperature for 30 minutes, gel filtration was performed on Sephadex G-200 using glycine-HCl buffer (0.5 M, pH 3.4) as the eluent. Into each fraction tube, 0.1 ml of 1 M Tris-HCl buffer (pH 7.2) was added beforehand. The amylase-lacking fraction between the first and second protein peaks and the normal sized amylase fraction were concentrated and mixed. The mixture was eluted by Sephadex G-75 (ø2.5×100 cm).

Enzymo-immunoelectrophoresis: To investigate the binding of amylase with immunoglobulin, electrophoresis was performed using agarose film as a support, and the formation of immunoprecipitin lines with the specific antisera (anti-human IgG, IgA and IgM immunoglobulins sheep sera, Hyland Go.) was observed. The precipitin lines were stained with blue starch at 37°C for 60 minutes.

Immune precipitation method: To investigate the binding of immunoglobulin with amylase, 2 to 40 μl of the patient's serum was added to 0.1 ml of each of the anti-human-immuno-globulin sheep sera. After standing at room temperature for 24 hours, the mixture was centrifuged at 3,000 rpm for 10 minutes and the amylase activity of the supernatant was measured. After the precipitate was washed three times with physiological...
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Fig. 4. Enzymo-immunelectrophoresis of the patient's serum. There was obvious amylase activity in the specific precipitin line between anti-human IgA and patient's serum. Weak amylase activity was found only in the IgA specific precipitin line between anti-whole-human serum sheep serum and patient's serum. The stained precipitin lines of this photograph are contrasted by penciling.

Fig. 5. Immuno precipitation of patient's serum. The solid lines show supernatant amylase activity and the broken lines the amount of protein in the precipitate.

saline, it was dissolved in 2.4 ml of 0.05 M glycine HCl buffer (pH 3.4) and the amount of protein was measured by 280 nm absorbancy.

RESULTS

For about six months, the patient's serum showed consistently high amylase activity, ranging from 3,148 to 4,420 IU. The Cam/Ccr showed a clearly low value of 0.09%.

In electrophoresis of the patient's serum, P-type was 12.4% (391 IU) and there was no S-type. Abnormal amylase (87.6%, 2,757 IU) was found in the globulin fraction slightly toward the anode from the origin (Fig. 1).

In gel filtration using Sephadex G-200, human serum protein produces three peaks. The amylase of a gallstone patient's serum was eluted after the third protein peak (Fig. 2A). However, in the patient's serum, most of the amylase activity was found between the first and second protein peaks, and amylase activity in the other fractions was low (Fig. 2B). When the patient's serum was acidified and underwent gel filtration, the elution pattern was the same as that of the control serum (Fig. 2E). When the mixture was again neutralized and the protein was subjected to the same gel filtration, the amylase activity again
shifted to the macromolecular protein fractions (Fig. 3). From these results, it was thought that the amylase was freed from the macromolecular substance when the serum was acidified and this change was incompletely reversed. The amylase in the patient’s saliva and concentrated urine was of normal size (Fig. 2C and D).

In enzymo-immunoelectrophoresis, no amylase activity of the specific precipitin lines between the patient’s serum and the anti-human immunoglobulins was found for IgG and IgM. There was amylase staining only in the specific precipitin line with anti-IgA. In the case of the patient’s serum and anti-human-whole-serum, amylase activity was found only in the IgA specific precipitin line (Fig. 4).

The results of immune precipitation method, and the precipitation between the patient’s serum and anti-human immunoglobulin (anti-IgA, anti-IgM and anti-IgG) are shown in Fig. 5. When the amount of antiserum was constant and the amount of patient’s serum added was increased, the amount of protein precipitated increased but the supernatant amylase activity decreased slightly in the case of anti-human IgA. In the case of anti-human IgG, the amount of precipitated protein and supernatant amylase activity increased up to 6 μl of patient’s serum added, but at 8 μl and over, the amount of protein precipitated decreased and the supernatant amylase activity increased. In the case of anti-human IgM, the amount of protein precipitated and the supernatant amylase activity both increased in proportion to the amount of patient’s serum between 10 μl and 40 μl added, but with under 10 μl, the increase in the amount of protein precipitated was less than that of the supernatant amylase activity.

**DISCUSSION**

Chronic hyperamylasemia without renal disease was first reported by Sachar et al. in 1956. Wilding et al. reported that the amylase was eluted between the 19S and 7S protein fractions on Sephadex-G 100 and assumed that the binding protein for amylase was gamma-macroglobulin because the phoresis of the macromolecule was in the fast gamma-slow beta globulin position in electrophoresis. Berk et al. designated this condition as macroamylasemia and postulated three possibilities: (1) polymerization of normal amylase, (2) binding of normal amylase and immunoglobulin or another protein, and (3) binding of normal amylase with a large non-protein molecule.

The case reported here was suspected clinically as a case of macroamylasemia because of continuous hyperamylasemia and a remarkably low Cam/Ccr.

Levitt et al. reported that the Cam/Ccr is useful as a screening test for macroamylasemia because the values of the Cam/Ccr are always low in macroamylasemia. The low Cam/Ccr indicates that the molecular weight of macroamylase is greater than 160,000. Molecules of this size are not filtered in appreciable quantities in the glomerulus. However, Berk et al. reported three cases which had low Cam/Ccr but no macroamylasemia. They concluded that the renal excretion capacity is greater for P-type amylase than for S-type amylase since there was an increase in S-type amylase in three cases, or that there was a decrease in the in vivo inactivation of S-type amylase. Therefore, the low Cam/Ccr dose not always mean the presence of macroamylase. However, by Cam/Ccr, which is simple to measure, should still be useful.

In 1968, Levitt reported a case of macroamylasemia with the 11S-type amylase bound to IgA. This macroamylase was considered to result from the antigen-antibody reaction. However, the authors believe that it is better not to consider this as a real antigen-antibody reaction since bound immunoglobulin does not act as a blocking antibody. There have been reports of IgG-binding type macroamylasemia, and 7S-type macroamylasemia without binding to an immunoglobulin.
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Clinical symptoms\(^{13}\) of macroamylasemia vary from patient to patient. Therefore, the actual cause of this disorder is not clear at present.

Fridhandler et al.\(^{14}\) reported a binding substance in macroamylasemia, and found that the S-type amylase showed stronger affinity. The affinity was not investigated in the case reported here, but since no S-type amylase could be found in electrophoresis and there was only amylase activity bands in the P-type amylase fraction and the globulin fraction, it seems possible that S-type amylase becomes mainly macromolecular.

In column chromatography, it was found that the size of the macroamylase in this patient was mainly between 7S and 19S.

The results of immune precipitation and enzyemo-immunoelectrophoresis showed that macroamylase of the patient was bound to IgA. In the case of anti-human IgG, the amount of precipitated protein decreased but supernatant amylase activity increased at 8μl and over of patient's serum added: One of the reasons for this was thought to be the addition of a relatively smaller amount of anti-human IgG. However, the peak precipitate occurred with 6μl of patient's serum, and no decrease of amylase activity was shown from 2 to 6μl of patient's serum (Fig. 5).

Hyperamylasemia of macroamylase is not derived from the escaping of the enzyme from tissues as is typical in acute pancreatitis but is a disturbance in the amylase clearance from the blood. However, since macroamylase has enzyme activity in vivo, and the binding substances are not constant from case to case, macroamylasemia is a highly interesting disease.

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REFERENCES