An anomalous response to increased reaction temperature is described for two samples of macroamylase, as contrasted with normal and pancreatic amylases. A possible method of differentiating macroamylase by assays of activities at 45°C and 25°C is suggested. The ratios of activities found at these two temperatures for a normoamylasemic population are given.

Additional Keyphrases: macroamylasemia • normal values • acute pancreatitis • diagnostic aid • bound and free enzyme

Macroamylasemia results from the complexing of serum α-amylase (EC 3.2.1.1; α-1,4-glucan 4-glucanohydrolase) to a high-molecular-weight protein or proteins (macroamylase) and is characterized by hyperamylasemia and low urinary amylase clearance, associated with normal renal function. Recently a simplified detection method has been described for macroamylasemia, in which a small column of Sephadex G-100 is used to separate normal unbound amylase from macroamylase (1). Although this technique is a considerable advance on the methods previously used, its use is still time consuming and it cannot readily be applied to large numbers of samples.

We have investigated the temperature–activity relationships of serum amylase (both at normal and elevated activities) and of macroamylase. These studies suggest that the ratio of amylase activities at two different temperatures can be used to differentiate a case of macroamylasemia from other causes of hyperamylasemia (2). A high serum amylase activity generally means that the urinary amylase–creatinine ratio or amylase clearance must be determined as a preliminary to diagnosing macroamylasemia. The temperature–activity ratio that we describe can be applied directly to a hyperamylasemic blood sample without requiring a sample of urine, and it would therefore seem to be ideally suited to population screening, if our findings are confirmed by other investigators.

Materials and Methods

Amylase estimation. The substrate is a water-insoluble cross-linked blue starch polymer, available in tablet form as the "Phadebas Amylase Test" (Pharmacia AB, Uppsala, Sweden). This substrate is saturating over a serum amylase activity of 35 U/liter to more than 9000 U/liter (3). Routine amylase assays are carried out at 37°C according to the manufacturer’s instructions. Our normal range of activities at this temperature for a hospital population is 70 to 300 U/liter. Although an age- and sex-dependent variation has been reported (4), this has only a slight effect on the range. The same assay technique and incubation times were used for determinations at other temperatures.

Patients' sera. Sera from hospital patients with amylase activities ranging up to three times the upper limit of normal were used for determining the normal 45°C/25°C amylase activity ratio.

Temperature–activity curves for serum amylase from patients with macroamylasemia, acute pancreatitis, and normoamylasemia were determined over the range 15°–60°C.

Macroamylasemia was diagnosed by hyperamylasemia and a low urinary amylase clearance in the presence of a normal creatinine clearance, and confirmed by column chromatographic separation of the enzyme (1).

Results

Figure 1 shows that normal serum amylase and amylase from a case of acute pancreatitis increases its activity by a factor of four between 25°C and 45°C, and that there is a peak of activity at about 50°C. Serum from a proven case of macroamylasemia shows an eight-fold increase in enzyme activity over the same temperature range.

These findings suggest that the ratio of amylase activities at 25°C and 45°C should differentiate between sera with and without macroamylase. Accordingly, we determined the amylase activity–temperature ratio in a series of hospitalized individuals (Table 1). The coefficient of variation of this ratio is 5%, indicating the assay to be remarkably precise.

A useful modification, which can increase the number of samples that may be done per day, is to halve the volume of serum samples assayed at 45°C and to double the incubation time of the sample assayed at 25°C. The normal ratio of enzyme activities is decreased from about four to about one, which has two advantages. Firstly, hyperamylasemic samples incubated at 45°C usually require dilution before the absorbance is measured, but the smaller sample volume obviates this. Secondly, there is a log-log relationship between enzyme activity and the absorbance of the test solution in the Phadebas amylase assay. This means that ratios of absorbances and ratios of enzyme activities derived from these absorbances are not equivalent unless the absorbances are approximately equal. Thus, with our modification we can use ratios of
absorbances (thus saving time reading the calibration graph) without loss of test precision (Table 1). This ratio was next determined on sera obtained from a known case of macroamylasemia during a month (Table 1). The ratios are clearly different from those obtained from the hospital population, and we used this test in a pilot study of 80 sequential hospital patients whose blood had been sent to the laboratory for serum electrolyte estimations. In this pilot study, we obtained a sample whose serum amylose was 10 times our upper limit of normal and whose amylose temperature ratio was 5.5. This was a case of head injury in the Intensive Care Unit, with no apparent trauma of salivary glands or abdomen. His urinary amylose clearance was subnormal and macroamylase was demonstrable in his serum. Again, several determinations of this ratio indicated a significant increase over the hospital population’s ratio (Table 1).

Discussion

The differential effect of temperature on the activity of enzymes from different tissue sources (for example, heat stability of placental alkaline phosphatase (5)) or isoenzymes (for example, serum cholinesterase (6)) has been utilized many times, but we are not aware of this effect being used to differentiate bound and unbound forms of the same enzyme. As a possible screening test, this is considerably more rapid than the Sephadex G-100 column chromatography screen previously described (1), which was capable of separating four serum samples per hour by use of two columns. Our manual test is a considerable advance on this column method, as it can be operated at the rate of about 20–40 samples per hour. We attempted to automate this screening test on the AutoAnalyzer (7,8) but the ratios we obtained with these methods do not agree with those reported here by use of the Phadebas assay, which suggest that there may be an uncovering of active sites in the amylase molecule of the macroamylase complex as the temperature increases. Quantitatively, the doubling of the 45°C/25°C ratio for macroamylase as compared with normal serum amylose indicates the possibility that one active site of the bound amylose is uncovered.

The inflection about 45°C (Figure 1) in the normal curve suggests the presence of two molecular species, and is reminiscent of the pancreatic and salivary amylases separated from urine (9). It was necessary to dilute the sera from patients with pancreatitis because of the high reaction velocities encountered, particularly at higher temperatures, and the effect of the second isoenzyme may either be swamped or diluted out by the preponderance of the pancreatic species.

About 40 cases of macroamylasemia have been reported (1) and we now report another two cases (10), one of which has been detected by the application of the rapid screening test reported here. Macroamylase exists in blood in at least two forms. One form is known to be an amylose-IgA complex (11) while the other form appears to be a complex of amylose with an as-yet-unrecognized protein or proteins (12). We have not yet typed our two cases of macroamylasemia, but it seems likely that neither case will prove to be of the IgA complex type, because malabsorption is not a clinical feature. This proviso is an important one, because the type of molecule complexing with the amylose may alter the temperature activity ratios. Nonetheless, we have demonstrated that this screening test is effective for our two cases.

It has been suggested that macroamylasemia may occur more frequently than the small number of cases reported would lead us to expect (13). In this connection we took part in a survey of blood samples from a nonhospitalized population of 2109 non-fasted persons (ages 45–64 years). Less than 2% had mild hyperamylasemia (300–400 U/liter), and none of these cases had macroamylasemia on the basis of our test.

Whether this temperature-ratio test can constitute a valid screening procedure requires examination in a much larger number of macroamylasemia sera than the two cases we describe here. It is in the hope that this essential validation will be carried out that we present these preliminary findings.

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References