Relation of Age to Isoenzyme Pattern and Total Activity of Amylase in Serum

P. J. Bossuyt, R. Van den Bogaert, S. L. Scharpè, and Y. Van Maercke

Pancreatic and salivary isoenzymes of amylase were determined in serum from 70 subjects. Thin-layer gel/isoelectric focusing was used to separate the isoenzymes. Because other studies (J. Lab. Clin. Med. 90: 141–151, 1977) show that the major isoenzymes have isoelectric points between 5.8 and 7.2, we focused the sera on polyacrylamide gel plates with a pH gradient from 5.5 to 8.5. The separated amylase fractions were made visible by direct incubation with a commercially available dye–starch polymer. Isoelectric focusing proved to be convenient, precise, and reproducible, and it can be used as a routine analysis to detect even slight changes in serum amylase distributions. We found that the isoenzyme distribution is age dependent, whereas total amylase activity shows no correlation with age.

Additional Keyphrases: isoelectric focusing • pancreatic disorders • electrophoresis, polyacrylamide gel • age-dependent effect

Separating amylase (EC 3.2.1.1) into its isoenzymes has been found to be useful in differential diagnosis of hyperamylasemia. Human serum has three major amylase isoenzymes, one originating from the pancreas and two from the salivary glands (1). Changes in the activity and pattern of these organ-specific isoenzymes can reveal pancreatic and parotid disorders.

After trying to separate the isoenzymes by electrophoresis on different supporting media (2–4), we tried a thin-layer gel/isoelectric focusing technique. This separating technique, based on the one described by Rosenmund and Kaczmarek (5), gave excellent resolution. The normal isoenzyme distribution in serum is not a clearcut pattern; it is affected by genetic variation and may vary with geographical location of the patient (4). We found it worthwhile to investigate whether this pattern is also age-dependent. Previous studies have suggested that for children the pancreatic isoenzymes start developing at three months and increase slowly until the age of 10 to 15 years (6, 7). The age group older than 15 was never thoroughly examined. Our study involved the entire age range.

Materials and Methods

Samples. Serum, saliva, and pancreatic juice were collected from two healthy donors. The saliva, taken sublingually, was centrifuged to remove particles. The pancreatic juice was collected by cannulation.

Serum was taken from 54 healthy volunteers, two months to 78 years old, and from five patients with acute pancreatitis and two with parotitis.

Seven patients, selected without conscious bias from a hospital population, provided serum for a reproducibility test.

Isoamylase standard solutions were a gift from Pharmacia Fine Chemicals, Uppsala, Sweden.

When necessary, the various samples were diluted with distilled water until the final activity was within a range convenient for detection of isoenzymes. All samples were stored at −20 °C before isoenzyme fractionation.

Amylase assay. Total amylase activity was determined with the Phadebas (Pharmacia) amylase test in accordance with the manufacturer’s instructions. The substrate is a water-insoluble cross-linked starch polymer carrying a blue dye. It is hydrolyzed by α-amylase to form water-soluble blue fragments. The absorbance of the blue solution is a function of the α-amylase activity in the sample. Absorbancy was measured with a spectrophotometer (Model 34; Beckman Instruments, Inc., Fullerton, CA 92634).

Thin-layer gel-isoelectric focusing. We used a flat bed apparatus (FBE 3000) from Pharmacia. Ampholine PAG plates, 1 mm thick, from LKB, Bromma, Sweden, with a pH gradient from 5.5 to 8.5 were used as medium. The anodal and cathodal solutions were 0.4 mol/L Hepes [4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid] buffer and 10 mmol/L NaOH, respectively. Power for the system was supplied by the ECPS 2000/300 power supply (Pharmacia). The gel plates were prefocused for 45 min at a constant power of 25 W and cooled to 5 °C. During prefocusing, the excess fluid near the cathode, produced by electrodosmosis, was regularly removed with filter paper. After prefocusing, we applied 40-μL samples with a micropipet at the anodal side, no more than 10 samples per gel plate. Three hours was the optimal time for the run. The power supply was set at 25 W and 2000 V. After the runs were completed, the plates were rinsed with distilled water.

Staining of enzyme and densitometry. A 2-millimeter-thick rubber frame was placed on the gel surface. A mixture containing five Phadebas Amylase tablets dissolved in 10 mL of distilled water was poured on the gel. The plate was then incubated for 3 h at 37 °C in a moist chamber. After this time the gel was rinsed again with water to remove remaining particles. Amylase isoenzymes were scanned with a densitometer at 600 nm.

Results

Evaluation of the Method

We determined the isoenzymes of amylase in seven sera, collected from a hospital population. The differentiations were repeated several times on the same samples during a month. Between each experiment the sera were stored at −20 °C. As previously described, storage for up to 18 months at −20 °C does not affect the isoenzyme pattern (8), nor did we see any tendency to change in activity during one month.

Table 1 shows the results of those experiments. The CV calculated for each group of repeated assays varied from 3.0 to 8.9%. Evidently, our method was reproducible within 9%.

We determined the accuracy of our method by using standard solutions (from Pharmacia) of different mixtures of purified human pancreatic and salivary isoenzymes of known activity. The different standard solutions were assayed with the isoelectric focusing technique. We used diluted saliva as a sample containing only salivary isoenzymes. Figure 1 shows
Table 1. Reproducibility of Serum Amylase Differentiation (Seven Samples)

<table>
<thead>
<tr>
<th>Amylase activity (U/L)</th>
<th>Pancreatic (P) fraction %</th>
<th>SD</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 387</td>
<td>30.2</td>
<td>2.7</td>
<td>(8.9)</td>
</tr>
<tr>
<td>4 415</td>
<td>66.8</td>
<td>2.5</td>
<td>(3.8)</td>
</tr>
<tr>
<td>5 205</td>
<td>39.8</td>
<td>1.9</td>
<td>(4.7)</td>
</tr>
<tr>
<td>4 194</td>
<td>54.0</td>
<td>1.6</td>
<td>(3.0)</td>
</tr>
<tr>
<td>4 184</td>
<td>42.0</td>
<td>3.2</td>
<td>(7.6)</td>
</tr>
<tr>
<td>5 311</td>
<td>47.0</td>
<td>2.3</td>
<td>(4.8)</td>
</tr>
<tr>
<td>4 475</td>
<td>74.0</td>
<td>4.5</td>
<td>(6.1)</td>
</tr>
</tbody>
</table>

the correlation between the given concentration of S-type isoenzyme and the amount found by isoelectric focusing.

Identification of the Enzyme

a. Normal serum isoenzyme pattern. Our results confirm the presence of two major groups of isoenzymes as previously described by many authors (9-12). One group of isoenzymes, originating from the pancreas, has at most three components; the other group, originating from the salivary glands, has at most four components.

To check whether we were correctly interpreting the different groups, serum, saliva, and pancreatic juice were sampled from two adult donors. The saliva and pancreatic juice, which had a much higher activity, were diluted as previously described. The samples were separated during the same run. Figure 2 shows the location of the two groups of organ-specific isoenzymes. Because of the extreme dilution of the saliva and pancreatic juice, only the dominant peaks appeared on the gel. When more-concentrated samples were run, both pancreatic juice and saliva could be seen to consist of five to six isoenzymes.

b. Pancreatic isoenzyme. Serum from five patients with acute pancreatitis demonstrated an extra peak on the cathodal side, which could not be detected in normal serum (Figure 3). The total amylase activity of these sera exceeded 1000 U/L. Therefore the samples had to be diluted with distilled water before assay. The pancreatic fractions contributed 75 to 100% of the total amylase activity. Further investigation is needed to determine whether the location of this extra peak points to a specific type of pancreatic disease.

c. Salivary isoenzyme. In sera from the two patients with parotitis, almost no pancreatic isoenzyme could be detected (Figure 4). The salivary fractions, which contributed more than 80% to the total activity, consisted of three to four enzymes. Here once more, due to the high total amylase activity, the samples had to be diluted. We are aware of the fact that some minor fractions are diluted out, but we believe their relative contribution to be negligibly small.

Age-Related Effect

Sera from 54 ostensibly healthy volunteers were used for this study. Total amylase activity was measured in all samples except for six from the age group two months to one year, for whom the specimen volume was too small. Therefore only 48

Fig. 1. Correlation between salivary (S) fractions of standard solutions as given by the manufacturer and as measured with the isoelectric focusing technique

Fig. 2. Isoamylase pattern of (a) serum, (b) pancreatic juice and (c) saliva from the same donor. P stands for pancreatic, S for salivary in Figs. 2-4.

Fig. 3. Isoenzyme pattern of (a) control serum and (b) serum from patients with acute pancreatitis. The control serum was arbitrarily taken from the healthy population.
The control serum was arbitrarily taken from the healthy population sera were assayed. Figure 5 shows the total activity as a function of age. Instead of using individual values, we calculated the mean total activity for each decade, because the individual ages per decade were homogeneously distributed. The mean activity for the entire population was 151 (SEM 10) U/L. Age was not related to total amylase activity.

The isoenzyme pattern was determined for the whole population. The percent of P-type isoenzyme was expressed as a function of age per decade (Figure 6) except for the age group from two months to one year, which was considered separately.

The isoenzyme pattern of serum amylase was clearly related to age. After age 40 the contribution of the P-fraction to the total amylase activity decreases rather rapidly. Even by age 30 to 40, the value for the age group is already significantly different from the value of the age group 50-60 (p < 0.02; Student's t-test for unpaired observations). We didn't think genetic variation could have influenced the age distribution, since other authors (4) found this genetic variation in less than 2% of the population. Age variation for children was previously described in detail by other authors (6, 7). They found an increase in pancreatic isoenzyme until the isoenzyme value for adults is reached. Skude (6) reports that adult values are reached by 10 to 15 years of age; Otsuki et al. (7) report they are attained by two years of age. A detailed distribution of an adult population has not been reported. O'Donnell et al. (13) found differences for serum amylase between men and women. They used a completely different technique, involving an inhibitor for the salivary isoenzyme.

Males and females contributed almost equally to the population in our study. We investigated for any sex-related dependency of values, but found no evidence of it. The same distribution and the same mean total activity were found for the male and female population.

Discussion

Our purpose was to investigate whether human serum amylase and its isoenzyme distribution are related to age. Therefore a reliable method for separating serum amylase into its major isoenzymes had to be assessed first. After trying several methods, we finally settled on a thin-layer gel/iso-electrofocusing technique, which proved to be reliable. This allowed us to make an accurate isoenzyme differentiation. We thus were able to show that the isoenzyme pattern—but not the total amylase activity—is related to age.

Therefore, when the isoenzyme distribution is assessed as an index to pancreatic and salivary disorders, the patient's age should be taken into account. A distribution that is perfectly normal for a 30-year-old person could be suspect for a 70-year-old.

Moreover, if one considers the mean isoenzyme pattern over the entire population, pancreatic fractions of more than 50% should be considered as suspect. However, this does not mean that such a value is clearly pathological because other factors such as genetic and racial variation or a drug therapy that affects pancreatic function may change isoenzyme distribution.

References


Fig. 4. Isoenzyme pattern of (a) control serum and (b) serum from patients with parotitis

The control serum was arbitrarily taken from the healthy population

Fig. 5. Total amylase activity as a function of age

All values shown are expressed as the mean per decade ± SEM (n = 6). Dotted line: mean total activity for the entire population

Fig. 6. P-fractions of amylase as percent of total in function of age

All values expressed as the mean per decade ± SEM (n = 6)