Agarose Electrophoresis and Inhibitor Tests for Isoamylase Determination Can Give Complementary Clinical Information

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We report the presence of an extremely high proportion of "aged" amylase in the serum and cyst fluid of a patient with a pancreatic pseudocyst. A salivary amylase inhibitor test helped us to differentiate these "aged" pancreatic amylases from salivary fractions having a similar electrophoretic mobility.

Additional Keyphrases: P- and S-type amylase isoenzymes, "aged" amylases, macroamylasemia, amylase electrophoresis, amylase inhibition, pancreatic pseudocysts, pancreatitis, monitoring treatment

In the past decade, isoamylase assays have emerged as a useful diagnostic tool in a wide array of clinical situations, particularly in gastroenterology, gynecology, and pediatrics (1-9). This increased popularity was largely catalyzed by the concurrent availability of simplified electrophoretic techniques (1, 2, 4) and spectrophotometric assays that exploit salivary isoamylase-specific inhibitors (5) or precipitating antibodies (6). Using such an inhibitor assay, Koehler et al. (7) showed that routine isoamylase determination could change the clinical diagnosis in 20 to 40% of hyperamylasemic patients. Some comparative studies have advocated the use of inhibitor tests for clinical routine applications (8, 9), but other reports have documented that electrophoretic techniques yield more clinically useful information, especially in cases of acute pancreatitis (10), pancreatic pseudocysts (11), extrapancreatic tumors (3), and macroamylasemia (4).

It is the purpose of the present study to reconcile the two opinions by illustrating that the two types of assays are complementary rather than mutually exclusive and hence should be ordered simultaneously in those patients with pancreatic disease or hyperamylasemia, or both, where unambiguous interpretation of isoamylase patterns is difficult.

Materials and Methods

Total α-amylase (1,4-α-D-glucan 4-glucanohydrolase; EC 3.2.1.1) activities were determined with the Chem-Strate® enzymatic amylase reagent from General Diagnostics, Morris Plains, NJ 07950, with maltotetraose as the substrate. We electrophoretically separated isoamylases according to the method of Leclerc and Forest (4), using Special Purpose Electrophoresis Film® agarose gels (Corning, Palo Alto, CA 94306) as solid support. Densitometric tracings of the isoamylase zymograms and quantification of the different isoamylase fractions (termed according to ref. 4) were obtained by means of a Model R-112 scanning densitometer (Beckman Instruments Inc., Fullerton, CA 92634). The electrophoretic mobility of the patient's isoamylase fractions was compared with that of control sera run on the same gel and obtained from patients with acute pancreatitis (containing fractions P2 and P3), mumps (containing S2 and S3), and lung cancer (containing S2, S3, S4, and S5 as proven by electrophoresis and inhibitor tests), as well as from a normal person whose serum showed the common genetic variant P1. We never observed the so-called S1 band (4) in serum. We quantified salivary (S-type) and pancreatic (P-type) amylase fractions by means of an S-type-specific inhibitor, using the Phadebas Isoamylase® test (Pharmacia, Upsala, Sweden). Serum and pseudocyst fluid were stored at −20°C until assayed for isoamylase (within two weeks after sample collection). Reported, under these conditions there is no significant in vitro aging of amylase (11), as we also verified by observing that no "aged" amylase fractions could be detected, after storage, in samples that initially contained no such fractions.

Case History

A 45-year-old woman with a long-standing history of alcohol abuse, chronic pancreatitis, and diabetes mellitus was hospitalized because of back pain, weight loss (14 kg over the past five months), loss of appetite, and fatigue. Physical examination revealed a thin, anorectic patient with a painless epigastric mass. Relevant laboratory findings at admission included above-normal values for erythrocyte sedimentation rate (46 mm/h; normal reference interval 0-20 mm/h), fibrinogen concentration in plasma (9.04 g/L; ref. interval 1.50-4.00 g/L), leukocyte count (11 400/mm³; ref. interval 4300-10 000/mm³) pre-prandial blood glucose concentration (2.15 g/L; ref. interval 0.70-1.00 g/L), and percentage hemoglobin A1c (10.8%; ref. interval 4.8-7.5%). The iron concentration in serum was 0.37 mg/L (ref. interval 0.60-1.70 mg/L) and the iron-binding capacity of serum was 4.27 mg/L (ref. interval 2.25-4.20 mg/L). Serum amylase activity (75 U/L; ref. interval <85 U/L) and results of liverfunction tests were normal.

A plain film of the abdomen showed calcifications in the epigastric region. The presence of a 127 × 157 × 160 mm thick-walled pseudocyst, located in the body of the pancreas, was documented by ultrasonic examination and computed tomography scan. Endoscopic retrograde cholangiopancreatography showed a "rat-tail" stricture of the main pancreatic duct.

At laparotomy a large pancreatic cyst was found, which adhered to the posterior wall of the stomach. A cystogastrostomy was performed after 1 L of clear citrine cyst fluid was drained away. The latter contained 96 000 U of amylase per liter. No bacterial colonies appeared in a six-day culture of the fluid.

Isoamylase fractionation of the patient's serum (before operation) showed four equidistant and nearly equally important fractions: two common pancreatic-isoenzyme fractions (termed P2 and P3, according to ref. 4) and two atypical fractions (termed P4 and P5, according to ref. 4) migrating in the zone where salivary fractions are usually

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observed (Figure 1A) and accounting for about half of the total amylase activity. We documented the pancreatic origin of these atypical fractions by performing a Phadebas Isoamylase test, which indicated that 100% of the serum amylase was of pancreatic origin. Moreover, P4 and P5 were also observed in the pancreatic cyst fluid, where they accounted for more than 65% of total amylase activity (Figure 1B). The normal genetic variant termed P1 according to ref. 4 was not present in the patient's body fluids.

No postoperative complications were observed. One year after the intervention the patient was free of symptoms. Her diabetes mellitus was being treated by diet only. Preprandial blood glucose concentrations were within normal limits. Serum amylase activity had declined to 16 U/L, and no P4 or P5 fraction could be demonstrated. Both clinical and ultrasonographic examination failed to disclose evidence of an epigastric mass.

Discussion

Our observation illustrate the notion that post-translational modifications of amylase molecules within pancreatic pseudocysts can lead to the appearance of so-called aged amylase fractions (P4- and P5-type) in serum, which mimick the presence of salivary isoenzymes (II) or of other less-common atypical amylase fractions such as tumor-associated salivary-like amylases (3), post-translational modified salivary or salivary-like amylases ("aged" salivary amylases; see ref. 3 and Figure 1) or relatively sharp macroamylase bands (12) that consist predominantly of complexed salivary amylase (13). As successful drainage of pancreatic pseudocysts has been shown to coincide with the disappearance from serum of "aged" pancreatic amylase bands (II), recognition of such fractions in the patient's serum by electrophoretic methods provides us with a marker in serum for pancreatic pseudocysts, allowing one to monitor the efficiency of treatment. Moreover, the relative amount of P4- and P5-type amylase fractions at the time the diagnosis is made is likely to reflect the duration of the "aging" process of amylase within cyst fluid—and hence the lifespan of the pseudocyst itself—because the relative importance of these "aged" amylases has been shown to increase during in vitro incubation of cyst fluid (11).

The present report draws attention to the fact that—at variance with other published amylase zymograms of pancreatic cyst fluid (11)—P4 and P5 isoenzymes can constitute the predominant amylase fractions in cyst fluid and even in the serum of patients with pancreatic pseudocysts, if the interval of pancreatic fluid stasis, and hence of amylase aging, is sufficiently long. The thickness of the pseudocyst wall and the large pseudocyst size suggest that this condition was fulfilled in our patient. Incidentally, we also found very large proportions of P4- and P5-amylose (Gorus, unpublished results) in peritoneal fluid and serum from a patient with pancreatic ascites caused by a pancreatic pseudocyst (14).

The presence of significant amounts of pseudo-salivary "aged" amylase fractions might falsely suggest extrapancreatic involvement in patients with hyperamylasemia and (or) pancreatic disease, hence initiating unnecessary investigations. Indeed, although there are small differences in electrophoretic mobility between, respectively, P3 and S2, P4 and S3, P5 and S4, and P5 and S5 (see Figure 1), it remains difficult to ascertain unambiguously the pancreatic or salivary origin of the faster-moving isoamylase fractions by visually comparing the patient's isoamylase zymogram with that of known control sera, especially in those instances where heavy—and hence somewhat broadened—bands are present or where mixtures of pancreatic and salivary fractions are incompletely resolved. Furthermore, the presence of unusual genetic variants, macromolecular complexes, or post-translational modifications might complicate the picture. The need to discriminate between salivary and "fast" pancreatic amylases is further highlighted by the finding that salivary fractions have been shown to be frequently present in sera from hyperamylasemic patients catalogued on clinical grounds as "probably" suffering from pancreatitis (7), whereas P3 fractions are a common finding in cases of acute pancreatitis (10) while P3, P4, and P5 fractions are not seldomly observed in cases of pancreatic pseudocysts (11). Moreover, the presence of amylase fractions in serum that are electrophoretically indistinguishable from, or very similar to, salivary isoenzymes have been described in association with various non-pancreatic non-salivary disorders, including various tumors (3). Hence, the additional clinically useful information conferred by the electrophoretic isoamylase assay in patients with pseudocysts can only be fully appreciated if the pancreatic origin of the fast-moving P4 and P5 bands can be documented. In the case we describe here this was achieved by comparing amylase zymograms from pancreatic pseudocyst fluid and serum and—more conveniently—for routine purposes—by using an S-type amylase-specific inhibitor test.

We conclude that the latter technique and agarose electrophoresis are not mutually exclusive but rather are complementary. While electrophoretic techniques appear superior to inhibitor tests, especially for the diagnosis of macroamylasemia (4), acute pancreatitis (10), pancreatic pseudocysts (11), and extrapancreatic tumors (3), an inhibitor test can help in establishing the actual origin of salivary-enzyme-like fractions seen in the zymogram. Alternatively, direct identification of isoamylase fractions by visual

**Fig. 1.** Denisotometric tracings of isoamylase zymograms

(A) Patient's serum before operation; (B) Cyst fluid collected during the intervention. The arrows indicate the position of reference isoamylase fractions (termed P1, P2, P3, P4, P5, S2, S3, S4, and S5, according to ref. 4), determined by comparison with the zymograms of control sera run on the same gel and obtained from patients with acute pancreatitis (containing P2 and P3), mumps (containing S2 and S3), and lung cancer (containing S2, S3, S4, and S5; the latter two fractions probably represent "aged" salivary amylases), as well as from a normal person displaying the uncommon genetic variant P1. The so-called S1 fraction (4) was never observed in serum.
inspection of electrophoretic patterns might be facilitated by using the method of Massey (10), which achieves better resolution in the P3—S2 zone. It remains, however, to be demonstrated whether that method allows a complete separation of more anodically migrating fractions such as P4, P5, S3, S4, and S5. Finally, this case report reinforces the contention (15) that isoamylase determinations can be clinically useful even in the absence of hyperamylasemia.

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References