Survey of α-Amylase Activity and Isoamylases in Autopsy Tissue

Richard O. Whitten, Wayne L. Chandler, Michael G. E. Thomas, Kathleen J. Clayson, and James S. Fine

We quantified total amylase and its isoenzymes in 22 different human tissues obtained at autopsy. Isoenzymes were separated by use of wheat-germ inhibition (WI) and electrophoresis on cellulose acetate (CA) and agarose (AG). Mean (±SD) total activity was highest in salivary glands (parotid 1710 ± 897 U/g, submandibular 605 ± 354 U/g), and pancreas (258 ± 137 U/g). All other tissues contained 100- to 1000-fold less amylase. As assessed with WI, pancreas, jejunum, liver, placenta, testis, skeletal muscle, and spleen contained more than 90% pancreatic isoamylase. Salivary glands and thyroid contained more than 90% salivary isoamylase. All other tissues contained a mixture of the two isoenzymes. CA and AG often produced different results. For both CA and AG the most common pancreatic isoforms were P2 and S1. Salivary gland homogenates demonstrated a band migrating in the P3 position on CA. We conclude that both types of amylase isoenzymes can be found in tissues other than salivary gland and pancreas, but that their low total amylase concentrations diminish their clinical importance.

Increased amylase (α-1,4-glucan-4-glucanohydrolase, EC 3.2.1.1) activity is observed in serum in some disease states (1-3). Amylase assays are widely used as a diagnostic adjunct, often in difficult clinical situations such as the differentiation of acute abdominal pain. Separation of amylase isoenzymes by selectively inhibiting salivary type isoenzymes, by electrophoresis, or by chromatographic techniques increases the diagnostic specificity of this enzyme (4-8). Kehler et al. (5) have shown that 20-40% of diabetics in cases of hyperamylasemia could be changed as a result of isoamylase determination.

To date, an extensive survey of the quantity and type of amylase present in human tissues has not been done. The purpose of this study was to further elucidate the potential sources of α-amylase isoenzymes and isoforms in major body tissues obtained at autopsy. To separate amylase isoenzymes and isoforms, we used the widely available techniques of wheat-germ inhibition (WI) and electrophoresis on cellulose acetate (CA) and agarose (AG) support media.

Materials and Methods

We obtained 107 specimens of 22 different tissue types at autopsy. Only one specimen of each tissue type was taken from a single autopsy. Postmortem intervals ranged from 5 to 40 h. Tissue selection was based on potential clinical relevance and included parotid and submandibular salivary glands, pancreas, stomach, duodenum, jejunum, ileum, colon, lung, fallopian tube, uterus, ovary, placenta, testes, heart, skeletal muscle, kidney, bladder, liver, gall bladder, spleen, and thyroid gland. Placental tissue was obtained postpartum. The number of samples of each tissue type is shown below in the caption of Figure 1. Adipose tissue and blood were carefully removed and the specimens were frozen at -70°C. The tissues were prepared as described previously (9). There was no evidence of disease as determined by clinical history and gross pathological examination in any of the organs obtained.

The total amylase activity assay, WI assay, and CA and AG electrophoretic procedures were performed exactly as described in the accompanying article (9).

Results

Total Activities

Mean total amylase activity, shown in Figure 1, was highest for the parotid gland (1710 ± 897 U per gram wet weight of tissue), followed by submandibular gland (605 ± 354 U/g), and pancreas (258 ± 137 U/g). Of the remaining tissues, none had mean total amylase activities greater than 5 U per gram of tissue. There was much variability among individual specimens within a tissue type, and it did not correlate well with the postmortem interval (see below).

Wheat-Germ Inhibitor Studies

Mean activities for pancreatic and salivary isoamylases as determined by WI are shown in Figure 2. Pancreas, all portions of the small bowel, placenta, kidney, liver, testis, skeletal muscle, and spleen contained >90% pancreatic-type amylase. In contrast, the salivary glands and the thyroid gland contained >90% salivary-type amylase. Heart sam-

1 Department of Laboratory Medicine, SB-10, University of Washington, Seattle, WA 98195.

* Please address reprint requests to this author.

* Nonstandard abbreviations: WI, wheat-germ inhibition; CA, cellulose acetate electrophoresis; AG, agarose electrophoresis; P, pancreatic (isoform); S, salivary (isoform).

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samples contained approximately 75% pancreatic isoamylase. The remaining tissues contained roughly equal amounts of each type of isoamylase.

Electrophoretic Studies

The nomenclature of amylase isoforms is confusing, and in our accompanying paper we define a system that is consistent and method dependent (9). According to this system, P3 and P5 are the most anodal pancreatic isoforms on CA and AG, respectively, and are probably equivalent.

The electrophoretic data presented below are available in tabular form, in more detail, upon request from the authors. Tables available include the proportion of cases displaying each band, by tissue type, for both CA and AG, and the mean percentage of the total that each band comprised for each tissue type (when that particular band was present), for both CA and AG.

Most of the activity in salivary glands was present in the S1 bands on both CA and AG. S1 represented 68% and 74% for parotid and submandibular gland on CA and 86% and 96% for parotid and submandibular gland on AG, respectively. Band patterns for both salivary gland tissues were complicated. Parotid glands had up to five bands on CA and up to four bands on AG, all of which were inhibited by WI by more than 50%, confirming their salivary origin. On CA, all parotid glands demonstrated S1 and S2 bands. 80% had an S4, and only 20% showed an S3. On AG, all parotid showed an S1, 60% showed an S2, and only 20% showed S3 or S4 bands. All samples from submandibular glands contained both S1 and S2 bands on CA, and on AG all showed an S1, but only 44% showed an S2. Neither method demonstrated S3 or S4 bands for the submandibular glands.

In addition to the typical salivary bands described above, about 80% of both salivary gland tissue samples showed a band that migrated in the P3 position on CA. This band was inhibited by WI, and we therefore classified it as a salivary isoenzyme. The activity of this band constituted about 10% of the total activity, representing approximately 170 U/g for the parotid glands and 60 U/g for the submandibular glands. An analogous band was not seen on AG.

The band having the greatest amylase activity from pancreatic tissue homogenates was P2 on CA (89% of the total), and P4 on AG (87% of the total). CA demonstrated only one other band, P1, in 29% of the samples. No P3 bands were present in any pancreatic sample using CA. On AG, all the samples showed a P4, 14% and 21% showed P1 and P2, respectively, and none showed P3 or P5 bands.

Thyroid gland was the only tissue other than the salivary glands that contained >50% S-type activity. The electrophoretic pattern of the thyroid homogenates was remarkable by both methods. In addition to the standard bands, all samples showed an amorphous smear containing faint, diffuse bands and migrating anodal to all other bands. Often >50% of the total amylase activity from this tissue was contained in this smear.

In other tissues, portions of the gastrointestinal tract have the highest total activity, and all segments showed P-type bands, with P2 being the most common on both CA and AG. For all segments, the majority of the amylase activity was contained in P2 on CA, but it was scattered through all of the P-type bands on AG. All stomach samples and a quarter of the bowel samples showed S-type bands on CA, S1 being the most common. None of the bowel or stomach samples showed S-type bands on AG.

The organs with the next highest total activity—lung and fallopian tube—showed both types of isoamylase on CA; P2 and S1 were the most common bands. On AG, lung showed both types of isoamylase, but fallopian tube showed only P-type bands.

Ovary, heart, gall bladder, kidney, uterus, and bladder showed a mixture of P- and S-type bands on CA, but only P type bands on AG. For CA, the P-type amylase activity was greater than S-type in most of the samples, with P2 containing the most.

The remainder of the tissues had less than 0.5 U/g total activity, making detection of bands difficult by either electrophoretic method. Placenta, for example, showed no bands by either method, even when the wells were maximally loaded with tissue extract. Liver showed a P-type band in one of six samples on CA and no bands of any type on AG. Testis occasionally showed bands of each type on CA, but no bands on AG. Skeletal muscle demonstrated an S-type band on one of four samples on CA and a P-type band on one of four samples on AG. Spleen showed only P-type bands by both methods.

Postmortem Interval and Amylase Activity

The amylase activity of individual tissues did not significantly change with increasing postmortem interval between time of death and time of tissue harvest. Pancreas and salivary glands, the tissue types of which we collected the most samples (14 of each), showed no correlation between total amylase activity and increasing postmortem interval; \( r = -0.21, P = 0.48 \) for pancreas, and \( r = 0.47, P = 0.09 \) for salivary glands. Moreover, the types of bands present electrophoretically did not show a consistent changing pattern. The percentage of amylase activity in P2 bands in pancreas did not show a significant change with increasing postmortem interval; \( r = 0.09, P = 0.18 \). The percentage of total amylase in S1 and S2 bands also showed no significant change with increasing postmortem interval; the slopes of regression analysis lines were not significantly different from zero: \( 0.00007 \pm 0.0022 \) and \( -0.00008 \pm 0.0007 \), respectively.

Discussion

Isoamylase types and quantities may change depending on the source of the enzyme: unsecreted amylase from healthy vs diseased organs, secreted isoamylase in the gut lumen vs the mouth, serum isoamylase in health vs disease. Unfortunately, there is no standardized preparation of amylase isoenzymes from any of these sources. In our study, we

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saw considerable variability in total amylase activity and in isoamylase band patterns within a given tissue type. However, this is not attributable to lability of the amylase enzyme during the postmortem interval of 5 to 40 h. The pancreas and bowel are notorious for their rapid and variable autolysis, and this process may account for some of the variations in amylase activity seen in many tissue samples. This problem cannot be overcome in an autopsy-tissue study. But amylase is known to be a stable enzyme (12), and our data support this fact.

The correlation between results by our electrophoretic isoamylase separation methods was generally poor. In our accompanying report (9) we showed that AG is not as precise as CA, and the detection limit for AG is not as good, especially for salivary isoamylase bands. Very few tissues contained salivary isoamylase bands of any type by AG. Most tissues contained an S1 or S2 band on CA, but these bands were present in only four tissues by AG. Three clinically important sources of amylase—the bowel, lung, and fallopian tube (2–4, 6, 7, 20)—showed marked differences when studied by the three different techniques. Thus, the isoamylase pattern found in the serum after injury to these tissues may differ greatly, depending on the technique used.

In contrast to certain previous studies (15, 16), our results indicate that the presence of certain pancreatic isoforms is not specific for a pancreatic source. Although the pancreatic form is present in the greatest concentration in the pancreas, alternative tissue sources could play an important role in the interpretation of amylase isoenzyme results in certain clinical situations. Using the tissue isoform concentrations from our study we calculated a rough estimation of serum amylase activity after injury to other organs. For example, necrosis of 300 g of jejunum could release 3.4 U of amylase per gram, or about 1000 U. Assuming a plasma volume of 2.5 L, 100% release of the enzyme from the tissue, and appearance of 50% of the enzyme in plasma, the resulting increase in serum amylase activity could be as much as 200 U/L, and most of the amylase released would be of the pancreatic type. An average 12 g of testis, on the other hand, if entirely necrosed by torsion, would release only 0.14 U/g, or 1.7 U. Thus, with the same assumptions of plasma volume and distribution the resulting increase in amylase activity in serum would be only 0.34 U/L, well below the upper reference range limit for our clinical assay (136 U/L). We emphasize that many assumptions are tested in these calculations, and that these numbers may not represent actual amounts of amylase found in the serum coincident with disease of these organs. More clinical research is needed to determine percentages released, volume of distribution, differences in production in various pathological conditions, and other variables.

Particularly important is our finding of a salivary isoamylase migrating in the P3 position on CA. If salivary gland releases this isoform into the blood when injured, the presence of this band on CA could be misinterpreted as evidence of pancreatitis, because a P3 band in serum has been considered quite specific for this condition (10, 13, 14).

Whether or not amylase is present in human liver has been controversial (3, 17, 18). Barros et al. (19) have implied that liver disease leads to hyperamylasemia. Many of their study patients had viral- or alcohol-induced liver disease. The amylase present in their patients' sera was predominantly salivary type by isoelectric focusing. We found little or no amylase in the liver. Evidently the amylase in the patients' sera studied by Barros et al. was from a source other than the liver.

Unlike previous studies (17, 20), we found pancreatic isoamylase activity in fallopian tube tissue by all three methods. Perhaps salivary type activity can be induced in this organ in physiological or pathological states such as blastocyst implantation or neoplastic change. A comparison of isoamyloses from normal and neoplastic tissues and quantitative studies of fallopian-tube amylases in various stages of ovum implantation would be helpful.

Amylase has also been found in amniotic fluid (21), although no correlation was seen between amylase concentrations in this fluid and in the corresponding maternal serum. Our studies suggest that the placenta is not the source of the amylase in amniotic fluid.

The anodal smear seen following electrophoresis of thyroid samples may be analogous to "aged" amylase present in pancreatic pseudocysts, which, according to Warshaw and Lee (22), migrates more anodally. They postulate that post-translational changes such as deglycosylation and deamination may be responsible and may occur non-enzymatically when amylase is "incubated" in the pseudocyst fluid. If amylase is secreted into the thyroid follicular fluid, a similar process may occur there.

In summary, we conducted a comprehensive survey of amylase activity and isoenzyme type in major human tissues. This study gives strong support to the widely held assumptions that the pancreas and salivary glands are the most important sources of serum amylase. However, both P and S isoamyloses are found in many other tissues.

References
7. Swenson EE, King ME, Malekpour A, Maucl KL. Serum amylo-1


