Comparison of Human Pancreatic and Parotid Amylase Activities on Different Substrates

Doris J. Stiefel and Patricia J. Keller

The specific activities of highly purified preparations of human parotid and pancreatic amylase on several different soluble and insoluble starches were compared. The ratio of parotid to pancreatic activity varied with the physical nature of the substrate. With all soluble starches, irrespective of source, parotid amylase exhibited a higher specific activity than did pancreatic amylase; the reverse was true for an insoluble chromogenic starch (Amylose Azure). The variation in enzyme-substrate interaction supports previous indications of configurational differences between the two amylases. The observed organ-specific characteristics according to organ of origin may have value in determining relative parotid and pancreatic amylase activities in body fluids under normal and pathological conditions, thereby helping to clarify their functional and clinical significance.

Additional Keyphrases: soluble and insoluble starches • enzyme activity

The enzyme α-amylase (α-1,4-glucan-4-glucanohydrolase, EC 3.2.1.1) has been extensively studied not only for reasons of its role in starch digestion in various mammalian species, including man, but also for its potential as an indicator of pathological conditions (1). Amylase is synthesized primarily by the salivary glands and the pancreas, and these organs are considered to be the principal sources of the amylases that are present in other body fluids, notably serum and urine (2-6). The possible contribution of additional amylases, from fallopian tube and liver, has not been completely established, but their chromatographic similarity to the pancreatic and salivary enzymes, respectively, has been reported (6). General uncertainty remains with respect to the function and significance of serum and urinary amylases, their normal activities, and alterations in these activities, both nonspecific and individual, in diverse conditions and disease states (1, 5-9). For these reasons, more precise methods of differentiating the amylases have been sought.

The nonidentity of human salivary and pancreatic amylase has become increasingly evident on the basis of a number of molecular and enzymatic findings, notably differences in electrophoretic mobility (3, 10-12); chromatographic and gel filtration profile (6, 11); molecular weight, carbohydrate content, and the accessibility of reactive sulfhydryl groups (13). An additional approach to establishing organ-specific differences has been made by comparing the ability of the enzymes to digest different starch substrates.

Meites and Rogols (14) reported that the amylases in human biological fluids can be divided into two classes. Group A, the amylase present in secretin-stimulated duodenal fluid, pancreatic extract, and serum from patients with pancreatitis, digested cornstarch faster than potato starch; Group B, the amy-
lase present in saliva, normal urine, and serum, usually digested potato starch faster than cornstarch. Because the samples tested were not pure, the validity of such differences is uncertain. Although noting some exceptions, Hall et al. (8) also observed a relatively greater activity by pancreatic amylase than salivary amylase toward cornstarch but concluded that pancreatic amylase is the predominant type in normal serum. However, in the latter study an insoluble cornstarch was compared with a soluble potato starch and the findings therefore did not resolve the question of whether the reversal in activity ratio was due to variation in plant source or to difference in the solubility of the substrate.

In the present study we have used highly purified preparations of human parotid amylase and human pancreatic amylase, to determine whether these could be differentiated on the basis of their activity toward various starches and to clarify the nature of such differences where present.

Materials and Methods

Amylase Samples

Crystalline human parotid amylase was prepared by the method of Kauffman et al. (15). Pancreatic amylase was purified from lyophilized human pancreatic juice as reported previously by us (14).

Amylase Assays

Amylase activity toward soluble starches was assayed by the dinitrosalicylic acid method of Fischer and Stein (16), except that the assay was done at 30°C and a buffer consisting of 20 mmol of sodium phosphate and 10 mmol of NaCl per liter, adjusted to pH 6.9, was used. The amylase reaction was stopped at the end of the usual assay period of 3 min, during which it is linear with time. Specific amylase activity was defined as the ratio of absorbance at 540 nm to enzyme concentration. The latter was calculated from the absorbance at 280 nm, by use of the value A_{280}^{1cm} = 23.3 (17).

Activity toward an insoluble chromogenic starch derivative was determined by the method of Hall et al. (18). After the reaction was stopped, the undigested substrate was removed by centrifugation at 7000 × g for 12 min. Enzyme activity was measured spectrophotometrically at 595 nm.

Starch Substrates

The following substrates were used:

- Cornstarches
  (b) A commercially modified, phosphate-derivatized, and cross-bonded waxy maize starch, "Versa-Stabe" No. 4832 (CPC International, Inc., Argo, Ill. 60501).

- Potato starches
  (a) "Noredux" starch (Gane's Chemical Works, Inc., New York, N.Y. 10017). This product was used without further modification.
  (b) "Aroostookrat" starch (A. E. Staley Manufacturing Co., Decatur, Ill. 62525). This was the same material as used in the investigations of Meites and Rogols (14) and was donated by these authors. This starch was acid treated in the same manner as the cornstarches.
  (c) Glycogen. This substrate, of shellfish origin (Mann Research Laboratories, Orangeburg, N.Y. 10962), was modified as for cornstarches except that the acid treatment was for 18 h at 35°C.

All substrate solutions were freshly prepared in a concentration of 10 g/liter of assay buffer. The potato and cornstarches were dissolved by heating; glycogen was dissolved at 35°C.

Specific activity of the enzymes on "Noredux" starch was used as the standard for comparison.

Results

In Figure 1 the specific activities of the two human amylases on the natural soluble starches are compared. Except for glycogen, which was less readily hydrolyzed; negligible differences in amyloysis rates were observed between the various natural starch products. Similar activities were also noted toward the commercially modified phosphate-derivatized cornstarch, "Versa-Stabe." For both enzymes there was only a small difference between their respective abilities to digest potato starch as opposed to cornstarch, with slightly more activity displayed by each enzyme toward the potato starch. At no time was a
reversal of the parotid/pancreatic activity ratio found for any of the soluble substrates; parotid amylase always exhibited a higher specific activity than did the pancreatic enzyme.

In contrast, Figure 2 shows that when assays were done with an insoluble chromogenic starch, Amylose Azure, such a reversal in activity ratio did occur, with relatively greater activity being displayed by pancreatic amylase. Although the substrate-to-enzyme ratio in the reaction mixture was about 10-fold greater for the insoluble starch than for the soluble starch, in both cases activity was measured under conditions such that it was linearly related to time. Substrate concentration therefore was not a limiting factor, and variation in quantity of substrate was not responsible for the difference in activity ratio.

The ratios of parotid to pancreatic amylase activities observed with the soluble and insoluble starches are shown in Table 1. The reversal in activity of the two enzymes toward the insoluble substrate is in agreement with the findings of Hall et al. (8).

**Table 1. Effect of Variation in Source of Substrate on Specific Activity of Human Amylases**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Parotid</th>
<th>Pancreatic</th>
<th>Parotid</th>
<th>Pancreatic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Soluble starch</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potato, “Noredux”</td>
<td>0.93</td>
<td>0.62</td>
<td>1.50</td>
<td></td>
</tr>
<tr>
<td>Potato, “Aroostocrat”</td>
<td>0.92</td>
<td>0.57</td>
<td>1.61</td>
<td></td>
</tr>
<tr>
<td>Corn, “Argo”</td>
<td>0.83</td>
<td>0.55</td>
<td>1.51</td>
<td></td>
</tr>
<tr>
<td>Corn, “Versa-Stabe”</td>
<td>0.84</td>
<td>0.57</td>
<td>1.46</td>
<td></td>
</tr>
<tr>
<td><strong>Glycogen</strong></td>
<td>0.48</td>
<td>0.38</td>
<td>1.27</td>
<td></td>
</tr>
<tr>
<td><strong>Insoluble starch</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn, “Amylose Azure”</td>
<td>0.58</td>
<td>0.86</td>
<td>0.68</td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

Our experiments on the highly purified enzymes demonstrate a relatively higher specific activity by human parotid amylase on all soluble substrates tested; in contrast, human pancreatic amylase has relatively more activity on an insoluble starch. Apparently the determining factor in substrate preference by the two amylases is the physical nature of the substrate rather than the plant source per se.

In previous studies with the same highly purified enzyme preparations, we observed certain physicochemical differences between the human amylases (13). Thus, at both high and low temperature extremes pancreatic amylase was more labile than the parotid enzyme. Furthermore, the single free sulfhydryl group appeared to be more accessible for reaction in pancreatic amylase than in parotid amylase. These findings suggest that the pancreatic amylase molecule is less compact than the parotid amylase molecule.

The variation in enzyme-substrate interaction we report here further substantiates the concept of configurational differences between the enzymes. The presumably more compact structure of parotid amylase may tend to hinder the binding of this enzyme with an insoluble substrate, whereas such steric hindrance might not be encountered with the presumably more loosely structured pancreatic amylase molecule. The present indications of functional heterogeneity of the human amylases, reflecting differences at the structural level, are consistent with findings of other investigators. Aw and Hobbs (19) observed a greater inhibition of human pancreatic than parotid amylase by a given antiseraum. Fridhandler et al. (20) noted that a binding substance isolated from human serum displayed greater affinity for salivary amylase than for pancreatic amylase.

In conjunction with suitable controls, it would appear productive to use the origin-specific differences in the amylases with respect to specific activity toward different substrates as the basis for relatively simple diagnostic assays of the principal amylase types present in a given biological sample. Where inconsistencies are observed it might be fruitful to examine the relationship to other parameters, both molecular and clinical. We do not know whether the differences we observed between parotid and pancreatic amylase pertain equally to their individual isoenzymes. If these can be isolated in adequate quantities, similar tests might be of value in making such determinations and, should differences exist, in probing their clinical significance.

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**Fig. 2.** Comparison of the activity of human pancreatic (A) and human parotid (O) amylases, both at 2.0 µg/ml concentration, toward an insoluble substrate, Amylose Azure. Experimental details are presented in the text.
mitted by Dr. D. J. Stiefel in November 1971, in partial fulfillment of the requirements for a Master of Science degree from the University of Washington. It was presented in part at the 50th annual meeting of the International Association for Dental Research in 1972.

References