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COMPARATIVE STUDIES ON ELECTROPHORETIC MOBILITY AND IMMUNOGENICITY OF PANCREATIC AND PAROTID AMYLASES OF RAT

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Summary

1. The α-amylases (1,4-α-D-glucan glucanohydrolase, EC 3.2.1.1) of rat serum, urine, pancreas, parotid gland and liver were separated by electrophoresis on a cellulose acetate membrane. They were found to be of three different types: a parotid gland type, a pancreatic type and a liver type. Rat serum and urine contained parotid type amylase only.

2. Antisera were prepared in rabbits against purified rat pancreatic amylase and parotid amylase. In addition to strong reactions between pancreatic amylase and its antiserum and between parotid amylase and its antiserum, a weak cross-reaction was observed between parotid amylase and anti-pancreatic amylaseserum. Anti-parotid-amylase serum gave an immunoprecipitation line with rat serum and urine, but anti-pancreatic-amylase serum did not, indicating that the amylases in serum and urine originate from parotid amylase.

Introduction

Mammalian amylases are produced in the pancreas and the parotid gland and catalyze the conversions of starch, glycogen and dextrin to maltose. Malacinski and Rutter [1] reported many electrophoretic variants of amylase in vertebrates, and differences between the tryptic maps of rabbit pancreatic and parotid amylases. Moreover, Sanders and Rutter [2] reported significant differences between pancreatic and parotid amylases of rats in amino acid composition and behavior in immunodiffusion tests. Although amylase is an exocrine enzyme in the alimentary tract, it is also present in the serum. Serum amylase in rats has the same electrophoretic mobility as parotid amylase [3]. However, it is unknown whether its antigenicity is similar to that of pancreatic or parotid amylase.

This paper reports on the electrophoretic mobilities and immunological properties of the amylase preparations obtained.

Materials and Methods

Assay of amylase activity. Amylase activity was assayed with blue starch (Pharmacia, Uppsala, Sweden) as described by Ceska et al. [5].

Electrophoresis of amylase. As described before [6], electrophoresis was performed using cellulose acetate membranes. After electrophoresis the membrane was incubated for about 1 h in contact with an agar plate containing blue starch. The density of the stained membrane was measured with a Chromoscan (Joice, Loebel and Co., Ltd, Gasteshead, England) using a 620 nm slit beam.

Preparation of pancreatic and parotid amylases. Male Donryu-strain rats, weighing about 200 g were used. Pancreatic and parotid amylases were purified by a modification of the method of Vandermeer and Christophe [4]. After ammonium sulfate fractionation, rat pancreatic amylase was purified by gel filtration on Sephadex G-25, chromatography on DEAE-Sephadex A-50, and gel filtration on Sephadex G-100. Final chromatography on phosphocellulose resolved pancreatic amylase into two isozymes.

After ammonium sulfate fractionation rat parotid amylase was purified by gel filtration on Sephadex G-25 and repeated chromatographies on DEAE-cellulose, yielding a single peak with amylase activity.

Preparation of antibodies. Anti-pancreatic-amylase serum and anti-parotid-amylase serum were prepared as described before [7]. The titers of anti-pancreatic-amylase serum and anti-parotid-amylase serum for pancreatic and parotid amylases (1 mg/ml), were both 1 : 128. IgG fractions of the antisera were separated by ammonium sulfate precipitation.

Immunodiffusion test. Immunodiffusion was performed by a modification of the method of Ouchterlony [8], as described before [7].

Results

Electrophoretic patterns

Three types of amylase were distinguished by electrophoresis on a cellulose acetate membrane, namely parotid, pancreatic and liver types, as shown in Fig. 1. There was no difference in the mobilities of amylase in a pancreatic homogenate and in the purified preparation obtained from Sephadex G-100. The mobilities of parotid amylase in a homogenate and in the purified preparation obtained by DEAE-cellulose rechromatography were also the same.

The electrophoretic mobility of rat liver amylase is entirely different from that of pancreatic or parotid amylase. The nature of rat liver amylase was proved to be a complex of glycogen and parotid amylase as reported in our previous paper [9].

Both crude and purified pancreatic amylase gave broader bands than those of amylase of the parotid, serum and urine. Serum and urine amylases showed only one band corresponding to that of purified parotid amylase, although sometimes there was a minor component on the anodic side of this. Unlike in humans [6] no pancreatic amylase was found in rat serum or urine under

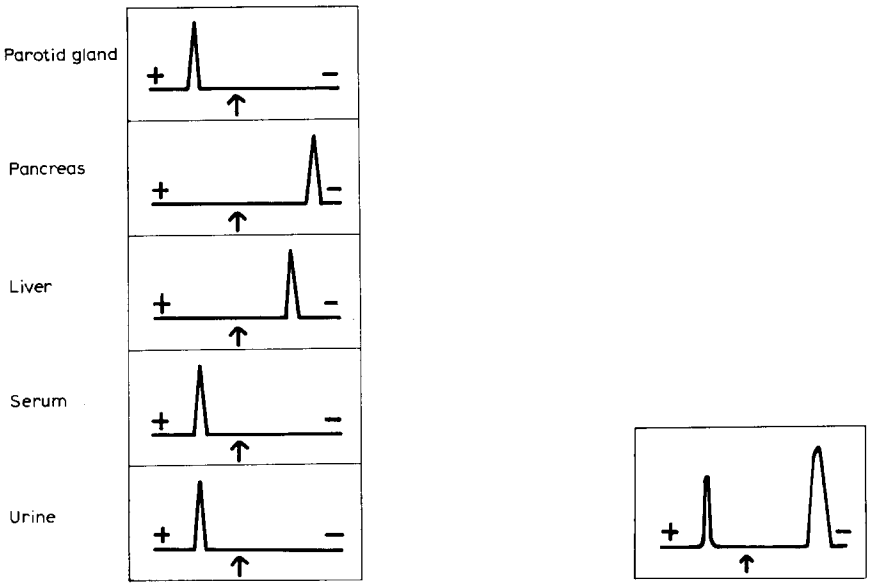

Fig. 1. Electrophoretograms of amylases of parotid gland, pancreas, liver, serum and urine. The conditions of electrophoresis are described in Materials and Methods. Arrows indicate the origin.

Fig. 2. Electrophoretogram of rat serum amylase after ligation of the pancreatic duct. The arrow indicates the origin.

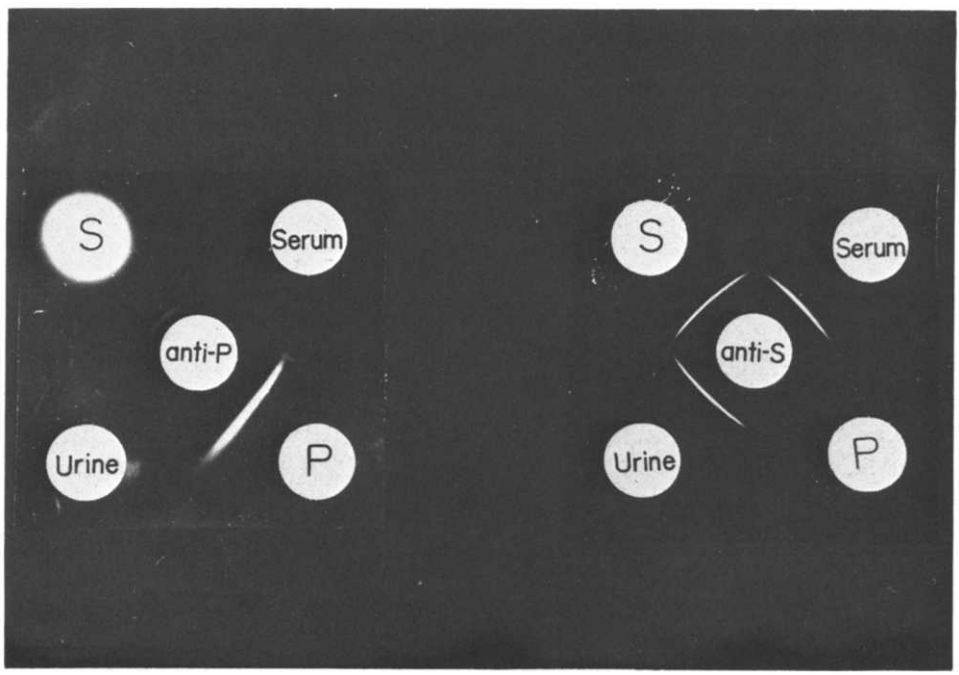

Fig. 3. Antigenicities of amylase isozymes. left: Anti-pancreatic-amylase serum (anti-P) was placed in the center well. Rat serum and urine and purified pancreatic amylase (P) and purified parotid amylase (S) were placed in the outer wells. right: Anti-parotid-amylase serum (anti-S) was placed in the center well and rat serum and urine and purified pancreatic amylase (P) and purified parotid amylase (S) in the outer wells.

normal conditions. However, when the pancreatic duct of rats was ligated or canulated, pancreatic type amylase appeared in the serum within 5 min, as shown in Fig. 2.

Immunodiffusion studies

Immunodiffusion studies were performed using a modification of the Ouchterlony method with anti-pancreatic-amylase serum (anti-P) and anti-parotid-amylase serum (anti-S). As shown in Fig. 3 (left), strong immunoprecipitation of anti-pancreatic-amylase serum (anti-P) against pancreatic amylase (P) was observed. Anti-pancreatic-amylase serum (anti-P) gave a weak reaction with rat parotid amylase (S). The amylases of rat serum and urine also gave weak reactions on prolonged incubation. As shown in Fig. 3 (right), anti-parotid amylase-serum (anti-S) gave strong immunoprecipitations with rat parotid (S), serum and urine amylases. Anti-parotid-amylase serum (anti-S) gave no immunoprecipitation with rat pancreatic amylase. There was no spur formation between rat parotid (S), serum and urine amylases, suggesting that the antigenicities of amylase in rat serum and urine are similar to that of rat parotid amylase.

Discussion

Immunological studies were made to determine the origin of rat serum amylase. It was found that only anti-parotid-amylase serum showed immunoprecipitation with rat serum and urine amylases. This agrees with the findings that rat parotid, serum and urine amylases had identical electrophoretic mobilities.

Human serum and urine contain two species of amylase isozymes, corresponding to pancreatic and salivary amylases, respectively [6]. Thus the question arises of why rat serum and urine contain only parotid type amylase, and no pancreatic amylase is found unless the pancreatic duct is ligated. The basal membrane of the acinar structure of the rat pancreatic cells may be strong enough to prevent leakage of amylase into the serum. Amylase in the serum may be derived only from the parotid gland. Evidence to support this possibility is that the antigenicity and electrophoretic mobility of parotid amylase are the same as those of rat serum amylase.

The fact that the antigenicities and electrophoretic mobilities of rat serum and urine amylases are similar to those of rat parotid amylase was observed with high reproducibility. Thus from the above considerations it seems clear that in rats serum and urine amylases originate from the parotid gland.

Acknowledgments

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