Glycosidases in Canine Pancreatic Secretion[1] (34994)

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In recent years, there has been considerable interest in lysosomal acid hydrolases. Among these enzymes, which are capable of degrading naturally occurring macromolecules (1), several glycosidases have been identified: neuraminidase (1), α-L-fucosidase (1), β-D-galactosidase (2), β-*N*-acetylgalactosaminidase (3) and β-*N*-acetylglucosaminidase (1). It has been assumed that the chief physiologic function of these lysosomal enzymes is to degrade within the cell substrates of extracellular or intracellular origins (1). Although lysosomal glycosidases have been found in extracts of various organs of different species, their presence in glandular secretions has not yet been investigated. The purpose of the study forming the basis for this report was to find out if glycosidase activities present in extracts of canine pancreas are also present in pancreatic juice.

Materials and Methods. Preparation of pancreatic tissue extracts. The pancreas of a dog was removed with sterile precautions after its vascular bed had been perfused with cold isotonic saline until the outflow was free of blood. The pancreatic tissue was then homogenized in cold isotonic saline (5 ml/g wet weight). Further disruption of the cells was achieved by sonic oscillation (5 min × 2, on ice). The tissue suspension was centrifuged in the cold for 16 min at 17,500 rpm. The supernatant fluid was withdrawn and assayed immediately for glycosidase activity as described below.

Collection of canine pancreatic juice. Duodenal fistulae were constructed in two dogs after the fashion described by Thomas (4). Pancreatic secretions, stimulated by a small meat ball given every 20 min were collected by a small glass probe introduced into the major pancreatic duct and connected by plastic tubing to a cylinder immersed in ice. Between collections, the dogs were maintained in good health on a standard kennel diet. At the end of each collection, the entire sample was dialyzed in the cold against 0.15 *M* NaCl for 15 hr.

Glycosidase assay conditions. Neuraminidase was assayed by incubating 0.2 ml of pancreatic juice or saline extract of pancreatic tissue with 0.1 ml of a solution containing 5 mg/ml of *N*-acetylneuraminyllactose and 0.5 ml of 0.1 *M* phosphate-citrate buffer, pH 5.0 for 60 min at 37°. The reaction was terminated by the addition of Na metaperiodate reagent, and the released free sialic acid in the mixture was estimated according to Warren (5).

α-D-galactosidase, β-D-galactosidase, β-*N*-acetylglucosaminidase, β-*N*-acetylgalactosaminidase, β-D-glucosidase, α-D-glucosidase, β-D-glucuronidase, α-D-glucuronidase, α-D-mannosidase, and α-L-fucosidase were assayed by incubating 0.4 ml of whole pancreatic juice or saline extract of pancreatic tissue with 0.1 ml of a solution of phenolic glycoside and 0.5 ml of 0.1 *M* phosphate–citrate buffer of a pH ranging from 2.5–7.5. The specific phenolic glycosides as well as the molarities in which these were used for routine assays are summarized in Table I. Reactions were terminated by the addition of 1.0 ml of 0.25 *M* Na_2CO_3 and the developed yellow color was measured immediately at

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TABLE I. Glycosidase Activities in Canine Pancreatic Tissue and Exocrine Secretion.

Glycosidases	Enzyme activities[a]	
	Pancreatic tissue[b]	Pancreatic secretion[c]
N-Acetyl-βD-glucosaminidase (*p*-nitrophenyl-*N*-acetyl-βD-glucosaminide, 10 m*M*)	2669.89 (pH 4.3)	22.57 (pH 4.3)
N-Acetyl-βD-galactosaminidase (*p*-nitrophenyl-*N*-acetyl-βD-galactosaminide, 2 m*M*)	500.00 (pH 4.3)	4.19 (pH 4.3)
αD-Mannosidase (*p*-nitrophenyl-αD-mannopyranoside, 10 m*M*)	274.26 (pH 4.2)	0.58 (pH 4.2)
αD-Galactosidase (*p*-nitrophenyl-αD-galactopyranoside, 10 m*M*)	1.21 (pH 6.5)	0
βD-Galactosidase (*o*-nitrophenyl-βD-galactopyranoside, 10 m*M*)	142.85 (pH 4.3)	0.26 (pH 4.3)
αD-Glucosidase (*p*-nitrophenyl-αD-glucopyranoside, 10 m*M*)	131.06 (pH 5.9)	0.14 (pH 5.9)
βD-Glucosidase (*p*-nitrophenyl-βD-glucopyranoside, 10 m*M*)	4.37 (pH 7.0)	0
βD-Glucuronidase (*p*-nitrophenyl-βD-glucuronide, 10 m*M*)	4.25 (pH 7.0)	0
αL-Fucosidase (*p*-nitrophenyl-αL-fucopyranoside, 2 m*M*)	70.09 (pH 5.9)	3.22 (pH 5.9)
Neuraminidase (*N*-acetylneuraminyllactose)	0	0

[a] (pH) = pH level of optimum enzyme activity.

[b] Velocities expressed as μmoles/liter-min/g of wet tissue.

[c] Velocities expressed as μmoles/liter-min/ml of pancreatic juice secreted in response to stimulation by a meal. Values in resting secretion were lower.

430 mμ. Enzyme activities were expressed as μmoles/liter-min of phenolic aglycon released from the substrate per gram of wet pancreatic tissue or per milliliter of pancreatic juice. The incubations were all done at 37°. To insure that the assays were done under conditions of zero-order kinetics, in each experiment one series of tubes was incubated for 15 min and another series for 30 min. In all experiments the degree of hydrolysis of substrate was less than 15%. Parallel substrate enzyme blanks were incubated under identical conditions and the extinction values thus obtained were subtracted from those obtained for the corresponding experimental digestion mixtures.

Under routine assay conditions, the coefficient of variation of the assay of 14 replicate samples of pancreatic juice for activities of β-*N*-acetylglucosaminidase, β-*N*-acetylgalactosaminidase, α-D-mannosidase, and α-L-fucosidase were 1.68%, 2.28%, 2.31%, and 1.57%, respectively. For the same four enzyme systems there was a linear relationship between enzyme activity and the volume of pancreatic juice assayed within the range from 0.2 ml–0.8 ml.

To estimate the influence of substrate concentration on velocity, the concentrations of several substrates were varied within the following ranges: *p*-nitrophenyl β-*N*-acetylglucosaminide, .05 to 1.0 m*M*; *p*-nitrophenyl β-*N*-acetylgalactosaminide, .04 to .4 m*M*; *p*-nitrophenyl α-D-mannoside, .05 to 1.0 m*M*; and *p*-nitrophenyl α-L-fucoside, .05 to 1.5 m*M*. Because of the limited solubility of the galactosaminide, estimations of velocity at higher substrate concentrations could not be

made. These kinetic studies were carried out only for those enzyme systems with high activities in pancreatic juice. They were not carried out on extracts of pancreatic tissue. All estimations of velocity in relation to substrate concentration were carried out at the previously determined optimum pH for the particular enzyme system and with the same individual sample of pancreatic juice. The effects of substrate concentration on enzyme velocity were analyzed by the method of Lineweaver and Burke using the mean of five experiments for the plot of $1/S$ against $1/v$.

Results. Data from routine assays are summarized in Table I. Of the enzyme systems studied, neuraminidase was the only one that could not be detected in pancreatic tissue. Nor was it present in pancreatic juice. α-D-Galactosidase, β-D-glucosidase, and β-D-glucuronidase were present in very low concentrations in pancreatic tissue extract and were not detectable in pancreatic juice. Good activities were found for the other enzyme systems in pancreatic extract and pancreatic juice. It will be seen from Table I that the pH optima in the juice and the tissue extract were identical for those enzyme systems present both in the tissue extract and pancreatic juice. Figure 1 illustrates the pH-activity curves for β-glucosaminidase, β-galactosaminidase, α-D-mannosidase and α-L-fucosidase in pancreatic tissue and pancreatic juice. The pH-activity curves for β-glucosaminidase and α-D-mannosidase in pancreatic juice and tissue had similar contours. There were slight differences in these curves for β-galactosaminidase and α-L-fucosidase.

Figure 2 illustrates the relationships between substrate concentration and velocity for the four enzyme systems in pancreatic juice: β-glucosaminidase, β-galactosaminidase, α-D-mannosidase, and α-L-fucosidase.

Discussion. Crude extracts of canine pancreas contain several glycosidase enzyme systems. Although no attempt was made to localize these enzyme activities in pancreatic lysosomal protein, they probably originate from pancreatic lysosomes since their pH optima closely follow those measured by others for lysosomal glycosidases. The fact that the glycosidases in pancreatic juice and pancreatic extracts have identical pH optima suggests that the tissue enzymes are secreted in pancreatic juice. Because the activities of the glycosidases in pancreatic juice are low by comparison with those of other pancreatic enzymes, such as trypsinogen and amylase, their presence in pancreatic juice might be considered an incidental phenomenon resulting, perhaps, from entrainment with other enzyme proteins. However, in other studies now being prepared for publication, we have found that the concentrations of β-glucosaminidase, β-galactosaminidase, α-D-mannosidase, and α-L-fucosidase in pancreatic juice increase in response to pancreatic stimulation by secretin–cholecystokinin to the same or greater degree than trypsinogen and amylase. With respect to a possible physiologic role of the glycosidases in pancreatic juice, it should be pointed out that the ability of a glycosidase to remove a glycosyl group from

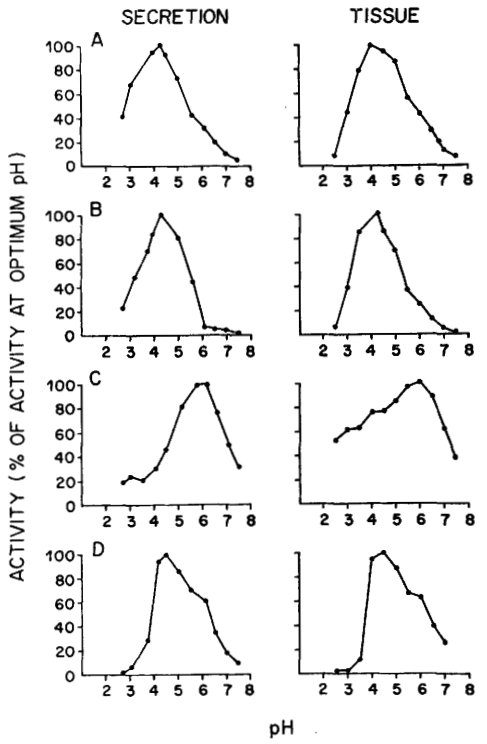

FIG. 1. pH-activity curves for β-glucosaminidase (A), β-galactosaminidase (B), α-L-fucosidase (C), and α-D-mannosidase (D) in pancreatic juice and pancreatic tissue. The values are plotted as percentages of activity at optimum pH.

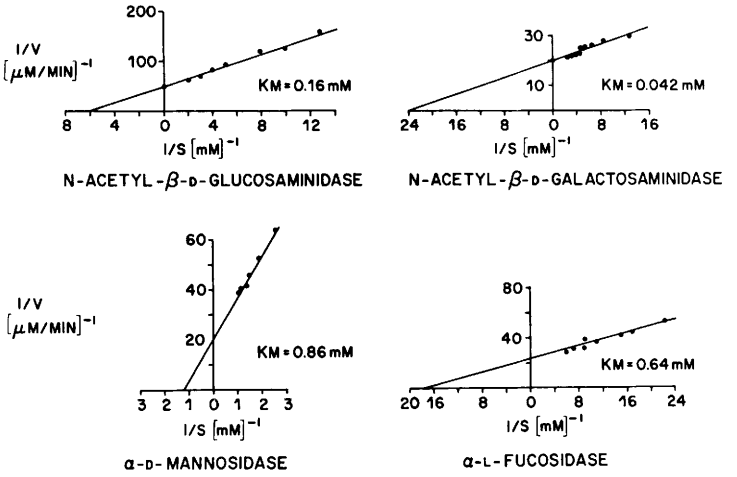

FIG. 2. Relation between S, the substrate concentration (mM), and v, the rate of hydrolysis (μmoles of nitrophenol liberated per liter-min per ml of pancreatic juice) by four glycosidases in canine pancreatic juice. Data are analyzed by the method of Lineweaver and Burke, plotting $1/S$ against $1/v$.

a synthetic substrate does not prove that the enzyme can release the same group from a naturally occurring large molecular substrate. However, we have also found that highly purified β-glucosaminidase from canine pancreatic juice releases *N*-acetyl-glucosamine from ovalbumin. It is, therefore, possible that these glycosidases belong to the physiologic complement of enzyme protein in exocrine pancreatic secretion.

Summary. Several glycosidases present in extracts of pancreatic tissue have been found in pancreatic juice. The corresponding enzyme systems in pancreatic juice and pancreatic tissue extract have identical pH optima and similar curves of pH activity. Velocity–substrate relationships of β-glucosaminidase, β-galactosaminidase, α-D-mannosidase, and α-L-fucosidase in pancreatic juice have been estimated.

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