Rat-Intestinal Dextranase

LOCALIZATION AND RELATION TO THE OTHER CARBOHYDRASES OF THE DIGESTIVE TRACT

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Extracts of the small-intestinal mucosa of different mammals, including man, possess dextranase activity (Adrouny, Bloom & Wilhelmi, 1957; Dahlqvist, 1961*a*, 1962). The dextranase activity seems to be sufficiently strong to enable a rapid utilization of ingested dextran (Bloom & Wilhelmi, 1952). The intestinal dextranase may also be of importance for the metabolism of dextran which has been administered parenterally as a plasma substitute, since this is known to be secreted into the gastrointestinal tract (Åberg, Bloom & Hansson, 1961). Dextran can, however, be hydrolysed in other parts of the body also, since several different tissues contain dextranase (Rosenfeld & Lukomskaya, 1956, 1957; Lukomskaya & Rosenfeld, 1958; Fischer & Stein, 1960).

The subject of the study reported here is the distribution and the specificity of the dextranase present in the digestive tract. Since the intestine of the rat has especially high dextranase activity (Adrouny *et al.* 1957) this animal was selected for the study.

MATERIALS AND METHODS

Determination of carbohydrase activities

Dextranase and amylase activities. The dextranase and amylase activities were measured by the methods used for the pig enzymes by Dahlqvist (1961*a*).

In the present paper the unit of amylase and dextranase activities has been defined as the activity liberating reducing groups corresponding to 1 μ mole of maltose (isomaltose)/min. at 37°, instead of 1 mg./hr. as in the previous investigation. This is in accordance with recommendations recently made by the joint Sub-Commission on Clinical Enzyme Units of the International Union of Biochemistry and the International Union of Pure and Applied Chemistry (Freeman, 1961). One unit defined in this way equals 21.6 units of those previously used.

Disaccharidase activities. For the assay of disaccharidase activities the tris-buffered glucose-oxidase reagent was used (Dahlqvist, 1961*b*). The incubation conditions were those used for the pig enzymes (Dahlqvist, 1960*b*, 1961*b*) but the unit is now defined as the activity hydrolysing 1 μ mole of disaccharide/min. at 37° instead of 1 mg./hr. One unit thus defined equals 21.6 of the units previously used.

Determination of protein

Protein determinations were performed with the method of Lowry, Rosebrough, Farr & Randall (1951), with the

modified 'reagent B' introduced by Eggstein & Kreutz (1955). A standard curve was prepared with freshly dissolved human serum albumin, kindly supplied by A. B. Kabi (Stockholm, Sweden).

Animal preparations

Rats of either sex, weighing 200–250 g., of the Sprague-Dawley strain were used.

Tissue homogenates. The rats were starved for 18–24 hr., with free access to water. Each rat was killed, the abdomen opened and the gastrointestinal tract taken out. The stomach was cut open and gently blotted to remove mucus. The small intestine, 70–80 cm. long, was divided into three equal parts. Since the small intestine was almost empty, it was not opened. The caecum and the large intestine were cut open, the contents removed and the mucosa was blotted. All tissues were weighed, and homogenized for 2 min. in an Ultra-Turrax homogenizer with 4 ml. of 0.9% sodium chloride/g. of tissue. The tube was chilled with crushed ice before and during homogenization. After the homogenates had been centrifuged at 2000g for 10 min. the opalescent supernatants were used for the determinations of carbohydrase activity and protein, in order to evaluate the distribution of the enzymes between the different organs. In some experiments a sample of the caecal contents was similarly homogenized.

Pancreatic juice. To collect pancreatic juice the lower end of the common bile-pancreatic duct was cannulated during ethernarcosis with a polyethylene tube. To avoid contamination with bile, this was removed through another cannula situated in the common bile duct near the liver. The animal was then placed in a small cage, allowing little movement, and the pancreatic juice was collected during 24 hr. in tubes chilled with solid carbon dioxide.

Subcellular fractions of mucosal homogenates. The small intestine was cut open and the mucosa removed with a piece of glass. From each rat 2–2.5 g. of small-intestinal mucosa was obtained. It was homogenized with 4 ml. of ice-cold 0.25M-sucrose solution/g. in a homogenizer of the Potter & Elvehjem (1936) type with a glass pestle. During homogenization and all subsequent steps the solution was chilled to prevent hydrolysis of the sucrose.

After removal of the nuclei and cell debris by centrifuging at 1000g for 10 min. in an International Refrigerated centrifuge, differential centrifuging was performed in a Spinco preparative ultracentrifuge (Hogeboom, 1955), with rotor no. 40. A fraction was first sedimented at 7000g for 20 min. ('mitochondria'), then resuspended in 0.25M-sucrose to give the original volume of the homogenate, and again sedimented at 7000g for 20 min. The combined supernatants were centrifuged at 100 000g for 45 min., giving a sediment ('microsomes') and a particle-free supernatant. Before analysis for carbohydrase activities the

sucrose was removed by dialysis against running tap water at 11° for 15–20 hr.

Solubilized mucosal enzymes. Small-intestinal mucosa was homogenized for 2 min. in the Ultra-Turrax homogenizer with 1 ml. of 0.2 M-sodium phosphate buffer (pH 7.0)/g. of tissue. During the homogenization the tube was chilled with ice. Then 1 mg. of crystalline trypsin was added/ml. of homogenate, and the tube was placed in a water bath at 37° for 2 hr. After this incubation the homogenate was chilled with crushed ice and the proteins were then precipitated by the addition of 3 vol. of 95% (v/v) ethanol, previously chilled to -16°. After centrifuging in an International Refrigerated centrifuge at 1000g for 10 min. the supernatant was discarded, and the sediment was taken up in water to give twice the volume of the original homogenate. The solution was centrifuged at 1000g for 10 min., and the sediment was discarded. The supernatant, which contains the solubilized carbohydrases, was dialysed for 16–24 hr. against water at 5°. It was stored at -16°.

Enzyme-fractionation methods

Anion-exchange chromatography. Diethylaminoethyl-cellulose (DEAE-cellulose) was prepared from Solka Floc cellulose powder SW 40A by the method of Peterson & Sober (1956). The product contained 0.8 m-equiv. of titratable alkaline groups/g. dry wt., titrated in 0.5 M-sodium chloride (Peterson & Sober, 1956). By ethylation as described by Porath (1957) the diethylaminoethylcellulose was converted into triethylaminoethylcellulose (TEAE-cellulose). This ion-exchanger was stored and used in its bromide form. For preparation of the columns the ion-exchanger was first stirred with 100 ml. of 0.01 M-phosphate buffer/g. for 30 min. The column was washed with 500–1000 ml. of the same buffer/g. before application of the protein.

Heat inactivation. Heat inactivation of the enzyme preparations was performed as described by Dahlqvist (1959a).

RESULTS

Distribution of carbohydrases in the digestive tract

Tissue homogenates. Homogenates of all parts of the small intestine of starved rats had considerable dextranase activity, whereas homogenates of the stomach, caecum and colon were poor in dextranase (Table 1). The dextranase activity was somewhat higher in the proximal two-thirds of the small intestine than in its distal third. Amylase activity,

in contrast, was present in considerable amount in all parts of the digestive tract. The disaccharidases, like the dextranase, were mainly localized in the small intestine. They were, however, not uniformly distributed along the small intestine. The invertase and isomaltase activities had a distribution similar to that of the dextranase, but the trehalase was considerably more concentrated in the proximal part of the small intestine, and the maltase activity was about equal in all three parts (Table 1). Nine rats investigated all showed essentially the same distribution of the carbohydrase activities.

Pancreatic juice. Rat pancreatic juice containing 40 mg. of protein/ml. had very powerful amylase (10 000 units/ml.), weak maltase (1–1.5 units/ml.), and no detectable dextranase, invertase, isomaltase or trehalase activity (less than 0.02 unit/ml.).

Caecal contents. Homogenates of the caecal contents of starved rats contained only low activities of the carbohydrases studied (Table 1).

Intracellular localization of carbohydrases

The major part of the dextranase, invertase and isomaltase activities of mucosal homogenates were recovered in the particulate fractions on differential centrifuging (Table 2). The three activities were distributed in a parallel manner, with the highest activity (units/mg. of protein) in the 100 000g fraction.

Solubilization and fractionation of enzymes

After digestion of homogenates of the mucosa of the small intestine with trypsin and purification by ethanol precipitation, 75% of the dextranase activity was recovered in soluble form together with the other carbohydrases. This preparation was used for a study of the separation of the enzymes by anion-exchange chromatography and heat inactivation. Trehalase activity was not followed in these experiments.

Anion-exchange chromatography. When the solubilized enzyme preparations were chromatographed on TEAE-cellulose, several peaks were obtained (Fig. 1). A column (1.0 cm. × 5.5 cm.) containing

Table 1. Protein content and some carbohydrase activities of different parts of the gastrointestinal tract of a starved rat, measured *in vitro* in tissue homogenates

Tissue	Weight of tissue (g.)	Experimental details are given in the text.						
		Protein (mg.)	Dextranase (units)	Amylase (units)	Invertase (units)	Maltase (units)	Isomaltase (units)	Trehalase (units)
Stomach	1.4	95	0.16	148	0.26	2.0	0.23	0.16
Small intestine								
Proximal third	2.4	273	4.7	271	8.0	39.1	9.1	10.0
Middle third	1.9	204	4.9	238	7.9	34.5	9.1	3.5
Distal third	2.1	179	2.8	850	3.2	34.4	6.3	0.37
Caecum	1.1	83	0.15	7.6	0.25	0.41	0.33	0.00
Colon	1.7	134	0.16	165	0.16	0.35	0.16	0.04
Caecal contents	1.7	51	0.12	3.3	0.42	0.06	0.19	0.03

Table 2. Intracellular distribution of the dextranase, invertase and isomaltase activities in subcellular fractions from 1 ml. of a homogenate of rat-small-intestinal mucosa

Experimental details are given in the text.

Fraction	Protein (mg.)	Dextranase (unit)	Invertase (unit)	Isomaltase (unit)
7 000g sediment ('mitochondria')	1.0	0.14	0.34	0.26
100 000g sediment ('microsomes')	0.9	0.19	0.52	0.30
Particle-free supernatant	1.8	0.13	0.44	0.19

Fig. 1. Gradient-elution chromatography of rat-intestinal carbohydrases on TEAE-cellulose. Experimental details are given in the text. (a): Δ , Buffer concentration; $\bullet$, protein concentration. (b): $\square$, Amylase; $\blacksquare$, dextranase. (c): $\bullet$, Maltase; $\circ$, isomaltase; $\circ$, invertase.

0.5 g. of TEAE-cellulose was used. A solubilized intestinal-mucosal preparation containing 6 mg. of protein, 1.8 units of dextranase, 16.2 units of amylase, 3.7 units of invertase, 17.1 units of maltase and 3.4 units of isomaltase, dissolved in 3.5 ml. of 0.01 M-phosphate buffer, pH 6.0, was applied to the column. The elution was performed at 20° with phosphate buffer, pH 6.0, of continuously increasing molarity, with a flow rate of 0.05–0.10 ml./min. Each fraction collected had a volume of 2.6 ml. In the effluent 86 % of the protein and 55–80 % of the carbohydrase activities were recovered.

The dextranase activity appeared as a single peak. The amylase activity was recovered in two peaks, both of which were separated from the dextranase (Fig. 1). The disaccharidase activities formed three different peaks. All of these three peaks contained maltase, two of them invertase and only one isomaltase. One disaccharidase peak appeared in the same fractions as the dextranase; this peak contained all of the isomaltase, about two-thirds of the invertase and one-fifth of the maltase activity recovered in the effluent.

Heat inactivation. The course of the heat inactivation of the carbohydrase activities of solubilized intestinal-mucosal preparations was followed

at temperatures varying between 45° and 60° and pH values between 5.0 and 8.0. The enzyme solution had previously been dialysed against the appropriate buffer (0.01 M-sodium acetate and 0.01 M-sodium phosphate buffers) for 20 hr. at 4°.

The dextranase, invertase and isomaltase activities under the conditions tested were all inactivated, following the course of a first-order reaction, i.e. when the logarithm of the amount of activity remaining was plotted against time a straight line was obtained (Fig. 2). The heterogeneity of the rat-intestinal invertase observed chromatographically thus could not be demonstrated in the heat-inactivation experiments. Under all conditions the inactivation of the dextranase and isomaltase activities ran closely parallel, but the invertase activity is exerted by an enzyme with a clearly different sensitivity to heat. At pH 5–7 the invertase was inactivated more rapidly than the dextranase and isomaltase activities, but at pH 8 the last two activities were more sensitive to heat than was the invertase. After heat-treatment at 50° for 1 hr. at pH 6.0 the invertase was completely inactivated, but 60 % of the isomaltase and dextranase activities remained.

The relation of the maltase activity to the other disaccharidase activities could not be evaluated from the heat-inactivation experiments, owing to the complicated course of the inactivation of maltase (Fig. 2). This course reflects the chromatographic heterogeneity of the maltase activity.

In some experiments the course of the heat inactivation of the amylase activity was also followed. The shape of the curve indicated the presence of two amylases with different sensitivities to heat.

DISCUSSION

In both rat and man the oral administration of dextran causes a rapid increase in blood sugar (Bloom & Wilhelmi, 1952), which has been assumed to mean that the polysaccharide is hydrolysed by a genuine intestinal enzyme and not solely by bacterial action (Bloom & Wilhelmi, 1952; Fischer & Stein, 1960). In agreement with this, dextranase was in the present study found to be localized mainly in the small intestine. The large intestine and the caecal contents had very little dextranase activity, which seems to rule out the possibility that the enzyme is of bacterial origin. The enzyme is probably formed in the small-intestinal mucosa, since the pancreatic juice did not contain dextranase. In homogenates of the small-intestinal mucosa the dextranase, like the disaccharidases, was mainly present in insoluble form, but could be solubilized by digestion with trypsin without appreciable loss of activity. The distribution of the dextranase activity in the

Fig. 2. Heat inactivation of the dextranase, invertase, maltase and isomaltase activities of solubilized rat-intestinal carbohydrase preparation (a) at 50° and at pH 6.0, (b) at 60° and at pH 6.0, and (c) at 45° and at pH 8.0. The buffer was 0.01 M-sodium phosphate. ○, Invertase; ●, maltase; ◐, isomaltase; ■, dextranase.

gastrointestinal tract is quite different from that of amylase. That amylase is distributed in all parts of the gastrointestinal tract is in accordance with the findings of McGeachin & Ford (1959). The separation of the dextranase and amylase activities by anion-exchange chromatography also clearly shows that they are exerted by different enzymes. The separation of the amylase in two different peaks does not seem to have been observed previously. The origin and specificity of these two amylases is as yet unknown.

Location in the small-intestinal mucosa is similar for the dextranase and the disaccharidases. The activities were not uniformly distributed along the small intestine. The trehalase activity was highest in the proximal third of the small intestine; the dextranase, invertase and isomaltase activities were strong in the whole small intestine, but highest in its proximal two-thirds; the maltase activity was about equal in all parts of the small intestine. The unequal distribution of these activities has previously been observed in the pig small intestine (Dahlqvist, 1961a).

The fractionation of the intestinal disaccharidases into several enzymes is in accordance with observations in pig (Dahlqvist, 1959a, b, 1960a) and man (Dahlqvist, 1962). The rat-intestinal invertase was fractionated into two components by anion-exchange chromatography, whereas in pig and man no heterogeneity of the intestinal invertase has been revealed. Although the different species hitherto investigated have a roughly similar set of intestinal disaccharidases, marked species differences may exist. Differences in specificity between the disaccharidases of the pig and man have been noted (Dahlqvist, 1962).

The fractionation methods separated the dextranase activity completely from the amylase and invertase activities, and also from the main part of the maltase activity. The trehalase activity was not studied in the fractionation experiments, but the distribution of the dextranase and trehalase activities in the small intestine shows that they belong to separate enzymes. In all the experiments, however, the dextranase and isomaltase activities closely followed each other.

These activities also were distributed in a parallel way in the digestive tract with a constant isomaltase:dextranase activity ratio of 2.0, which strongly suggests that the rat-intestinal dextranase and isomaltase are a single enzyme.

SUMMARY

1. The intestinal dextranase is confined to the small-intestinal mucosa. Like the disaccharidases it is chiefly particulate in mucosal homogenates, but can be solubilized with trypsin without appreciable loss of activity.

2. The distribution of the dextranase and the results of fractionation (by anion-exchange chromatography and heat inactivation) demonstrated that the dextranase is distinct from amylase, invertase, trehalase and at least the major part of the maltase activity.

3. Isomaltase, in contrast, was indistinguishable from the dextranase with a constant isomaltase:dextranase activity ratio of 2.0. It is suggested that these two activities are exerted by the same enzyme.

4. The amylase activity of mucosal preparations was found to belong to two different enzymes. The disaccharidase activities were exerted by a mixture of enzymes, roughly similar to those previously recognized in preparations from pig- and human-small-intestinal mucosa, but showing a species difference in containing two invertases.

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