A Comparative Study of Maltase & Glucoamylase in the Intestine of Various Animal Species

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Maltase and glucoamylase (\(\gamma\)-amylase) activities were determined in the small intestine of rat, rabbit, monkey, guineapig, chicken and pigeon. The distribution of these enzymes along the intestine and the effect of thermal inactivation at 55, 60 and 65°C were studied. The development of maltase, sucrase and glucoamylase with age was studied in the rabbit. Maltase-sucrase-invertase complex (maltase I) and maltase-glucoamylase complex (maltase II) could be conveniently separated by gel filtration on Sepharose 6B columns. Maltase I was absent at birth but became detectable on 21st day and thereafter increased to reach adult levels.

The presence of amylases in various tissues of mammals has been demonstrated for a considerably long time. The dietary starch and glycogen are hydrolysed by salivary amylase to a small extent but mostly by pancreatic amylase giving rise to a mixture of oligosaccharides and disaccharides. The oligosaccharides and undigested polysaccharides are hydrolysed to glucose by the intestinal glucosamylase also known as \(\gamma\)-amylase. The disaccharides are hydrolysed by specific intestinal disaccharidases to the corresponding monosaccharides. These digestive enzymes of the intestine are localized in the brush border of the epithelial cells and carry out the hydrolysis at the cell surface. That the disaccharidases are not uniformly distributed along the length of the intestine, has been shown in adult and suckling rats, hamster, monkey and the human, chicken, 6-day-old chick and the pig.

The heterogeneity of the intestinal maltase has been demonstrated by studying the rate of thermal inactivation in the monkey, chicken, pig and in the human.

In view of the above observations and the fact that relatively little information was available on the glucoamylase of the small intestine, a comparative study on the heterogeneity and distribution along the intestine of maltase and glucoamylase was undertaken. In the rabbit, a study of the development with age of maltase sucrase and glucoamylase has also been made.

Materials and Methods

Chemicals and enzymes — The following chemicals and enzymes were purchased commercially as indicated: maltose, sucrose, tris(hydroxymethyl)-aminomethane, d-glucosidase, glucose oxidase and peroxidase from Sigma Chemical Co., St. Louis, USA; starch and ethylendiamine tetraacetate acid from E. Merck AG, Darmstadt; Triton X-100 was a gift from Rohm and Has.

Other chemicals used were of analytical grade.

Preparation of homogenates — The animals were killed by decapitation (rat, Millardia mettardia; rabbit, Oryctolagus cuniculus; guineapig, Cavia; chicken, Gallus gallus; pigeon, Columba livia) or by anaesthetizing with nembutal (monkey, Macaca radiata) and the small intestine from pyloric to ileocaecal end was removed and chilled in ice. In the case of birds, the intestine from the gizzard end to the caecal end was removed. They were then washed in ice-cold 1.15% KCl, cut open longitudinally and the mucosa scraped with a blunt knife. A 20% (w/v) homogenate of the mucosa was prepared in 0.01M sodium phosphate buffer, pH 7.0.

For distribution studies, the intestine was cut into 10 equal segments. Mucosa was obtained from each segment, and was numbered, starting from the pyloric end. Homogenization was performed with a teflon homogenizer (rat, guineapig, chicken and pigeon) or the Sorvali Omni-mixer (monkey and rabbit). In each species, 2 individuals were used except the rat where 4 were used. For the developmental study in the rabbit, 2 or more individuals in each age group were used. With individuals below 21 days of age, homogenates of the whole intestine were used and with those above 21 days the mucosa was scraped and homogenized.

Assay of enzymes — Glucoamylase activity was assayed with starch as substrate by the measurement of the glucose formed by the glucose oxidase-peroxidase procedure of Dahlqvist using 0.5M tris-HCl. The reaction mixture (0.5 ml) contained sodium phosphate buffer, pH 6.0 (50 \(\mu\)moles), starch (8 mg), EDTA (0.5 \(\mu\)mol) and the enzyme. The reaction was stopped after 1 hr at 37°C by the addition of tris-glucose oxidase-peroxidase reagent and further incubated for 1 hr at 37°C. The reading was taken with a Klett-Summerson Colorimeter with a No. 42 filter. EDTA was added to inhibit \(\gamma\)-amylase activity of the homogenate and had no effect on the \(\gamma\)-amylase.

Maltase activity was determined as described earlier with the incubation mixture containing maltose (0.05M) and sodium phosphate buffer, pH 6.0 (0.1M final). For assays in the developmental study in the rabbit, sodium phosphate buffer pH 7.0,
which was found to be the optimum, was used. Sucrase was assayed in a reaction mixture similar to that for maltase but using sucrose as substrate.

Protein was determined by the procedure of Lowry et al.14 with crystalline bovine serum albumin as standard.

Enzyme units — One unit of glucoamylase activity was defined as the amount of enzyme required to produce 1 pmole of glucose per minute at 37°C with starch as substrate. One unit of maltase or sucrase was the amount of enzyme required to hydrolyse 1 pmole of substrate/min at 37°C. Specific activity was expressed as units/mg protein.

Results
Maltase and glucoamylase activities in whole intestinal homogenate — Maltase and glucoamylase activities were observed in all the 6 animal species studied (Table 1). The ratio of maltose to glucoamylase was highest in the rabbit (ratio, 9.63). In the case of monkey and guinea pig the ratio of the two activities is similar and in the range 4.0–4.8. In the rat and the pigeon, the ratio was around 2.0–2.5 and it was lowest in the chicken (ratio, 1.73).

Distribution along the intestine — All the animals showed a similar distribution pattern for maltase and glucoamylase. With the exception of the pigeon, maximum activities were found in the middle region of the gut corresponding to the jejunum and decreased gradually on either side of the jejunal region (Fig. 1). In the pigeon, maximum activity of maltase and glucoamylase was noted in the first 3 segments of the gut corresponding to the duodenum and proximal jejunum, decreasing gradually to very low values in the ileocecal end.

Heat inactivation — These studies were made with crude homogenates suspended in 0.01 M sodium phosphate buffer, pH 7.0 and suitably diluted to maintain a protein content of 2–3 mg/ml. Inactivation was done at 3 different temperatures namely 55, 60 and 65°C. The homogenate was maintained at each temperature for 1 hr, and aliquots withdrawn every 20 min were assayed for maltase and glucoamylase. In general, 3 species of maltieses could be distinguished by the difference in their heat sensitivity. These maltieses have been designated by earlier workers9 as maltase I, II and III in the order of increasing heat stability.

With the rat, guinea pig, chicken and pigeon, it was found that the glucoamylase closely parallels the maltase activity (Fig. 2). But in the monkey, as has already been reported13, and in the rabbit, all of the glucoamylase is inactivated along with maltase II, maltase I being free of any glucoamylase. That glucoamylase exists in association with maltase II, has been shown by the isolation and characterization of the maltase-glucoamylase complex in monkey, human and rabbit.15 Maltase I, which is labile at 55°C, has been shown to exist in association with sucrase and isomaltase.10,11

Development of maltase, sucrase and glucoamylase during growth in the rabbit — At birth, sucrase is absent whereas maltase and glucoamylase are present at very low levels, with a ratio of activities of maltase to glucoamylase around 3 (Fig. 3). The activities remain at the same low level up to the 21st day after birth at which time the sucrase begins to appear. Simultaneously with the appearance of the sucrase

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<th>Length of small intestine (cm)</th>
<th>Maltase</th>
<th>Glucoamylase</th>
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<tr>
<td>Rat</td>
<td>75</td>
<td>0.30</td>
<td>74.0</td>
</tr>
<tr>
<td>Rabbit</td>
<td>240</td>
<td>0.12</td>
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Fig. 1 — Distribution of maltase and glucoamylase along the intestine of adult animals of various species. The segments were numbered starting from the pyloric end.

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activity, the maltase and glucoamylase levels rise rapidly and reach maximal values around the 60th day. A steep increase (almost 10-fold) in specific activity is noted between the 21st and 26th days in the case of maltase and glucoamylase.

In order to estimate maltase I and II as a percentage of total maltase activity, a gel filtration on Sepharose 6B was carried out (Fig. 4). Unlike Sephadex, which shows a moderate interaction with maltase I and a strong interaction with maltase II (maltase-glucoamylase complex), the enzymes showed no

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**Fig. 2**—Stepwise heat inactivation of intestinal maltase and glucoamylase of adult animals of various species

**Fig. 3**—Development of maltase, sucrase and glucoamylase in rabbit during growth

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**Fig. 4**—Separation of rabbit intestinal maltase I and maltase II by Sepharose 6B chromatography. The papain solubilized intestinal extract obtained at various stages of development was fractionated on a Sepharose 6B column (2.3 x 43 cm) and 3 ml fractions were collected. The equilibrating and eluting buffer was 0.01 M K-phosphate, pH 7.0, containing 0.1M KC1. The fractions were assayed for maltase (•—•), sucrase (○—○) and glucoamylase (□—□) with 1-day old (A), 24 days old (B), 45 days old (C) and 6 months old adult (D) rabbits respectively.
interaction with Sepharose. Crude solubilized preparations from four different age groups with maltase/glucosamylase ratios varying from 3-10 were used. In the enzyme from 1-day old baby rabbits, almost all of the maltase activity can be attributed to glucosamylase, both the activities emerging as a single peak from the column. After the appearance of sucrose activity on about the 21st day, the maltase-sucrase complex (maltase I) steadily increases from 66% in the 24-day old animal to 80% in the adult, with a decrease in maltase II from 27 to 15% in the same period. It has been shown earlier by heat inactivation experiments that maltase I represents 65-70% of the total mucosal maltase in adult pig6 and monkey5 and about 80% in the human7.

Discussion

Maltase and glucosamylase activities are present in the intestines of all the species investigated. Maximum levels of both enzymes are present in the jejunum with the exception of the pigeon wherein maximal levels were present in the duodenum and proximal jejunum. The distribution of maltase shows a pattern very similar to that in other animals3,13,14. The distribution of glucosamylase has not been studied in any great detail with the exception of the pig15.

The development of maltase and sucrose in the rabbit is in accord with earlier reports on the development of α-glucosidases in various animal species16-19. The pattern in the human, however, differs from the pattern in other animals in that the adult levels of α-glucosidases are present at birth20-22.

To the best of our knowledge, the development of glucosamylase has not been systematically investigated earlier. However, the presence of low amounts of intestinal amylase and amylase in the newborn human23. In the present study, the pattern of development of glucosamylase was found to be very similar to that of maltase. The maltase/glucosamylase ratio, which is around 3 at birth, remains constant up to the 13th day, reaches a peak on the 60th day and then declines to the normal adult level of 10.

It is clear that glucosamylase is associated with a specific maltase isozyme (maltase II) as seen by thermal inactivation and Sepharose 6B filtration. Sucrase, on the other hand, is associated with maltase I (maltase-sucrase-ismaltase complex)24,25. A simple and rapid method for preparing rabbit intestinal glucosamylase in a homogeneous form is now available, and further work on the mechanism of action of glucosamylase is in progress.

Acknowledgement

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References