Studies on Mammalian Glucoamylases with Special Reference to Monkey Intestinal Glucoamylase

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(Received 15 December 1969)

1. Highly purified preparations of glucoamylase were obtained from liver, spleen and intestine of the monkey. The enrichment factor was lower for intestine (60-fold) compared with that of liver (1200-fold) and of spleen (2000-fold) but the final specific activities were of a similar magnitude. 2. The liver and spleen enzymes had maximum activity at pH 4.8 whereas the intestinal enzyme showed an optimum at pH 5.8. The \( K_m \) values for both starch and maltose with spleen and liver enzymes were higher than for the intestinal enzyme. With the intestinal enzyme, the \( V_{max} \) values were higher for both starch and maltose than those of the spleen and liver enzymes. 3. Gel filtration on Sephadex G-200 under identical conditions revealed that liver and spleen enzymes emerge from the columns much later than the intestinal enzyme. 4. Evidence is presented that the glucoamylase activity of the intestinal mucoza is exhibited by the maltase II fraction. 5. Tris, pentaerythritol and turanose inhibited glucoamylase from all the three tissues, but turanose inhibited the spleen and liver enzymes to a higher degree than the intestinal enzyme.

The role of the enzyme showing both acid \( \alpha-1,4\)-glucosidase (\( \alpha-D\)-glucoside glucohydrolase; EC 3.2.1.20) and glucoamylase (\( \alpha-1,4\)-glucan glucohydrolase, EC 3.2.1.3) activities in glycogenolysis in normal metabolism has been well documented (Rosenfeld, Lukomskaia, Rudakova & Schubina, 1959; Rosenfeld & Popova, 1962; Rosenfeld, 1964). Further support for its role came from studies on a type of glycogen storage disease, namely Pompe's disease, in which an absence of the enzyme was demonstrated (Hers, 1963; Baudhuin, Hers & Loeb, 1964; Illingworth & Brown, 1965). In the liver the enzyme is probably associated with lysosomes (Lejeune, Thinès-Sempoux & Hers, 1963). Reports on the partial purification of the enzyme from dog liver (Torres & Olavarria, 1964) and from rat liver and human kidney (Auricchio & Bruni, 1967; Auricchio, Bruni & Sica, 1968) have appeared. A highly purified preparation was obtained from bovine spleen by Fujimori, Hizukuri & Nikuni (1968).

The presence of a glucoamylase with an associated maltase activity was demonstrated in rat intestine (Dahlgqvist & Thomson, 1963) but was shown to have a different subcellular localization (Ruttloff, Noack, Friese, Schenk & Proll, 1967). Eggermont (1969) has shown the presence of a glucoamylase in human intestine.

Preliminary experiments on enzyme preparations from monkey tissues with Sephadex G-200 fractionation indicated that there was a striking difference between the glucoamylase from liver and spleen, and that of the intestine. A partial purification of the enzyme from the three tissues of the monkey was therefore attempted. A comparison of their properties is given in the present paper; a preliminary report has appeared (Seetharam, 1969).

EXPERIMENTAL

Materials and methods

**Chemicals.** The following chemicals were bought commercially as indicated: starch (E. Merck A.-G., Darmstadt, Germany); maltose (BDH Chemicals Ltd., Poole, Dorset, U.K.); glucose oxidase (type II, purified), horseradish peroxidase (type VI), \( \alpha \)-dianisidine, tris, bovine serum albumin (Sigma Chemical Co., St Louis, Mo., U.S.A.); turanose, Schardinger \( \alpha \) and \( \beta \)-dextrin and melezitose (Mann Research Laboratories, New York, N.Y., U.S.A.); pentaerythritol (J. and H. Berge, Plainfield, N.J., U.S.A.); bentonite (Amend Drug and Chemical Inc., New York, N.Y., U.S.A.); phenyl \( \alpha \)-D-glucoside (National Chemical Laboratory, Poona, India). Triton X-100 was a gift from Rohm and Haas Co., Philadelphia, Pa., U.S.A. Isomaltose was a pure sample kindly provided by Dr A. Jeanes, U.S. Department of Agriculture, Peoria, Ill., U.S.A. All the other chemicals were of analytical grade.

**Preparation of the homogenates.** Monkeys (*Macaca mulatta*) were anaesthetized with Nembutal before being
killed. The whole liver, spleen and the entire length of the small intestine were removed. The liver and spleen were cleaned and cut into small pieces and homogenized with 4 vol. of NaCl (25 mM) containing 1 mM-EDTA (hereafter referred to as NaCl-EDTA solution) in a Sorvall Omni-Mixer at 0–5°C for 5–10 min. The intestines were washed with ice-cold 1.15% KCl and then cut open longitudinally. The mucosa was scraped off and homogenized as described above with 4 vol. of 0.01 M-sodium phosphate buffer, pH 7.0.

Assay of enzyme activities. Glucoamylase activity in liver and spleen preparations was assayed essentially as described by Rutloff, Friese, Täufel & Täufel (1968) with starch as the substrate and measurement of the glucose formed by the glucose oxidase-peroxidase procedure of Dahlqvist (1961). The incubation mixture (final vol. 0.5 ml) contained sodium citrate-phosphate buffer, pH 4.8 (100 μmol), starch (5 mg), EDTA (1 μmol) and the enzyme (1–4 m-units). The reaction was stopped after 1 h at 37°C by heating the tubes in boiling water (2 min). The tubes were then cooled and 3 ml of tri-α-glucose oxidase-peroxidase reagent was added and incubated for 1 h at 37°C, and the readings were taken with a Klett-Summerson colorimeter with a no. 42 filter. EDTA was included in the assay mixture to inhibit the a-amylase activity of the homogenates. At the later stages of purification, the addition of EDTA was not essential.

The conditions for the assay of the intestinal enzyme were the same as described above except for the use of sodium phosphate buffer, pH 5.8.

Maltase was assayed as described by Swaminathan & Radhakrishnan (1965) by the procedure of Dahlqvist (1961). Both glucoamylase and maltase activities were measured at pH 4.8 in liver and spleen, and at pH 5.8 in the intestine.

Enzyme units. One unit of glucoamylase activity was defined as the amount of enzyme required to produce 1 μmol of glucose/min at 37°C; one unit of maltase activity was the amount of enzyme required to hydrolyse 1 μmol of maltose/min at 37°C. The specific activity is expressed as m-units/mg of protein.

Protein. Protein was determined by the procedure of Lowry, Rosebrough, Farr & Randall (1951) with crystalline bovine serum albumin as standard.

Purification of glucoamylase

From liver and spleen. All operations were carried out at 0–5°C unless otherwise stated. The same purification procedure was adopted for both the liver and spleen enzymes. The crude 20% homogenates in NaCl-EDTA solution were adjusted to pH 4.0 by the careful addition of 1 M-acetic acid and were centrifuged at 100000 g for 1 h. A portion (200 ml) of the supernatant fraction was heated at 60°C for 5 min at pH 4.0 and centrifuged to remove an inactive sediment. The supernatant fraction was adjusted to pH 7.0, and solid (NH₄)₂SO₄ added to give 30% saturation. The precipitate obtained on centrifugation was discarded and the supernatant fraction was raised to 60%, saturation and centrifuged. This precipitate containing the enzyme was dissolved in 35 ml of 1 mM-sodium phosphate buffer, pH 7.0, and exhaustively dialysed in the cold against 1 mM-sodium phosphate buffer, pH 7.0. A sample (30 ml) of the dialysed preparation was applied to a column (1 cm x 30 cm) of DEAE-Sephadex equilibrated with 10 mM-sodium phosphate buffer, pH 7.0. After being washed with 5 bed vol. of 10 mM-sodium phosphate buffer, the column was eluted stepwise with 5 bed vol. of 50 mM- and of 200 mM-sodium phosphate buffer, pH 7.0, respectively. The bulk of the enzyme was eluted with 200 mM buffer, and this fraction was concentrated by the addition of solid (NH₄)₂SO₄ to 90% saturation. The precipitate obtained after centrifugation was dialysed against NaCl-EDTA solution and then applied to a Sephadex G-200 column (2.4 cm x 27 cm) and eluted with NaCl-EDTA solution. The active fractions were pooled and used for kinetic studies.

The purification procedure employed for the spleen enzyme was the same as that employed for the liver enzyme, except that the heat treatment at 60°C was omitted.

By the above procedures the enrichment of the liver enzyme was 1200-fold with a recovery of 17%, and for the spleen enzyme, 1900-fold with a recovery of 11% (Table 1).

From intestine. A 50% (w/v) homogenate (300 ml) of the intestinal mucosa in 1.15% KCl was centrifuged at 100000 g for 1 h. The supernatant fraction was discarded and the pellet was suspended in 10 mM-sodium phosphate buffer.

### Table 1. Purification of glucoamylase from monkey liver and spleen

<table>
<thead>
<tr>
<th>Stage Description</th>
<th>Glucoamylase activity (m-units/mg of protein)</th>
<th>Maltase/glucoamylase specific-activity ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Liver)</td>
<td>(Spleen)</td>
</tr>
<tr>
<td>1 Homogenate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Acid treatment</td>
<td>2.5</td>
<td>2.6</td>
</tr>
<tr>
<td>3 Heat treatment</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>4 30-60% satd. (NH₄)₂SO₄</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 DEAE-Sephadex (pooled fraction)</td>
<td>21.8</td>
<td>31.0</td>
</tr>
<tr>
<td>6 Sephadex G-200 (pooled fraction)</td>
<td>143.0</td>
<td>86.0</td>
</tr>
<tr>
<td></td>
<td>2816.0</td>
<td>5000.0</td>
</tr>
</tbody>
</table>

* Maltase activity was separately determined for each fraction, as described in the Materials and Methods section, and the ratio calculated.
Table 2. Purification of glucoamylase from monkey small intestine

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
<th>Glucoamylase activity (m-units/mg of protein)</th>
<th>Maltase/glucocinamylase specific activity ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100000g sediment</td>
<td>139</td>
<td>4.2</td>
</tr>
<tr>
<td>2</td>
<td>Papain-solubilized supernatant</td>
<td>1050</td>
<td>4.9</td>
</tr>
<tr>
<td>3</td>
<td>50–80% -saturated (NH4)2SO4</td>
<td>1550</td>
<td>5.1</td>
</tr>
<tr>
<td>4</td>
<td>Bentonite-treated supernatant</td>
<td>3500</td>
<td>4.9</td>
</tr>
<tr>
<td>5</td>
<td>Heat treatment</td>
<td>4300</td>
<td>1.8</td>
</tr>
<tr>
<td>6</td>
<td>DEAE-Sephadex (pooled fraction)</td>
<td>8600</td>
<td>1.1</td>
</tr>
</tbody>
</table>

The above solubilized fraction (250 ml) was subjected to (NH4)2SO4 fractionation. The fraction precipitating between 50 and 80% saturation contained most of the enzyme activity. This fraction was suspended in 60 ml of 10 mM-sodium phosphate buffer, pH 7.0, and was dialysed against 1 mM-sodium phosphate buffer, pH 7.0, for 24h. To the dialysed fraction (55 ml) washed bentonite was added (10 mg of bentonite/mg of protein) and, after mixing, was left for 15 min and then centrifuged at 10000g for 20 min. The buffer concentration of the supernatant fraction containing the enzyme was adjusted to 5 mM and the solution was then subjected to heat treatment at 55°C for 5 min. An inactive precipitate was removed by centrifugation at 10000g for 20 min and the supernatant fraction was dialysed against 1 mM-sodium phosphate buffer, pH 7.0, for 24 h.

The dialysed fraction (38 ml) was applied to a column (1 cm x 15 cm) of DEAE-Sephadex A-50 equilibrated with 10 mM-sodium phosphate buffer, pH 7.0. The elution was carried out with a linear 0.01–0.1 mM-sodium phosphate buffer, pH 7.0, gradient (Varigrad, Technicon) with a flow rate of 10 ml/h. Fractions (3 ml) were collected and the enzymatically active fractions were pooled. The enzyme was purified about 60-fold with a recovery of 11% (Table 2).

The final purified glucoamylase fractions were essentially free of α-amylase activity as judged by assay of enzyme activity in undiluted fractions at various stages of purification by the 3,5-dinitrosalicylate procedure (Sumner, 1924) in the presence of tris (0.1 M) to inhibit glucoamylase activity (Seetharam, Swaminathan & Radhakrishnan, 1969). In liver, α-amylase activity was 5.3, 16.0 and 0 m-units/mg of protein respectively in the crude homogenate, pH4.0 supernatant and heat-treated supernatant fractions. All the α-amylase activity was destroyed when heated at 60°C for 5 min at pH 4.0. With spleen there was very little α-amylase activity at the crude-homogenate stage (2 m-units/mg of protein) and this was absent after the pH 4.0 treatment. In the intestinal mucosa over 90% of the α-amylase activity was removed together with the supernatant fraction of the homogenate (Seetharam et al. 1969) and the small amount of enzyme present in the pellet fraction was removed during purification. The activity in the crude pellet fraction, papain-solubilized fraction and 50-80% saturated-(NH4)2SO4 fraction was respectively 20, 80 and 0 m-units/mg of protein.

Isomaltase activity was determined at various stages of purification of the enzymes from monkey tissues by the procedure of Dahlqvist (1961) with tris-glucose oxidase reagent. With the 30–60% -saturated-(NH4)2SO4 fraction from liver, isomaltase activity was only 2% of the maltase activity, when a large amount of enzyme equivalent to 20–40 m-units of maltase was used. Similarly, in spleen the isomaltase activity of the (NH4)2SO4 fraction was only 4% of maltase activity. It was difficult to determine the isomaltase activity of the later fractions in view of the large volumes of the fractions required for assay. In the intestine the isomaltase activity of papain-solubilized and 50–80% -saturated-(NH4)2SO4 fractions was 10% of the maltase activity and decreased to about 0.3% after the bentonite and heat-treatment steps.

The significant purification of the liver enzyme was achieved in the Sephadex G-200 fractionation step. It is not clear whether there is a retardation of the enzyme fraction due to an enzyme–substrate type of interaction as suggested by Auricchio et al. (1968), or due to some other type of binding by the dextran gel. The spleen enzyme also behaved in a similar way. The intestinal enzyme, however, emerged near the void volume of the column. It was not affected by the isomaltase component of the fraction, since the papain-solubilized preparation had a much higher isomaltase activity than the liver and spleen fractions used for gel filtration. Although the enrichment factor for the intestinal enzyme was lower, the specific activity of the final preparation was higher than that of the liver and spleen enzymes because of the higher initial activity.

RESULTS

Glucoamylase activity in different tissues

Glucoamylase activity was determined in different tissues from the monkey. The intestinal mucosa showed the highest specific activity, whereas liver, kidney and spleen gave very low values (Table 3). When a homogenate prepared in 1.15% potassium chloride from the above tissues...
Table 3. Glucoamylase activity in various tissues of the monkey

<table>
<thead>
<tr>
<th>Organ</th>
<th>Glucoamylase activity (m-units/mg of protein)</th>
<th>(total units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestine</td>
<td>70</td>
<td>280</td>
</tr>
<tr>
<td>Liver</td>
<td>8</td>
<td>114</td>
</tr>
<tr>
<td>Kidney</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Spleen</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

was centrifuged at high speed (100000g, 1h) the bulk (90%) of the glucoamylase activity of liver, kidney and spleen was found in the supernatant fraction, whereas over 90% of the intestinal enzyme was in the sediment fraction, and this could be solubilized by treatment with papain.

Properties of the purified enzymes

The rate of hydrolysis of maltose or starch was proportional to the time of incubation (up to 1h) and to the protein concentration under the assay conditions employed for all three enzymes. The other properties studied are given below.

pH optima. With starch as substrate, the purified liver and spleen enzymes had maximum activity at pH 4.8 (Fig. 1). In a similar experiment (not shown in Fig. 1) it was found that the maltase activity paralleled starch hydrolysis. The purified intestinal enzyme exhibited an optimum at pH 5.8 with either starch or maltose as substrate.

Substrate specificity and $K_m$ values. The purified preparations (1–4m-units) of liver and spleen glucoamylase show very narrow substrate specificity, acting only on starch and maltose. With the quantity of enzymes used for the hydrolysis of maltose or starch, no activity was demonstrable towards dextran, sucrose, isomaltose, lactose and Scharndering $\alpha$- and $\beta$-dextrins. The purified intestinal enzyme acted on starch and maltose with only slight activity towards melizitose, turanose and phenyl $\alpha$-D-glucoside with quantities of enzyme equivalent respectively to 15, 30 and 15m-units of maltase.

The $K_m$ values for liver and spleen enzymes with either maltose or starch as substrate were similar in magnitude, but the corresponding $K_m$ values were much lower for the intestinal enzyme. The $V_{\text{max}}$ values were much higher for the intestinal enzyme (Table 4).

Inhibition studies. Tris, pentaerythritol and turanose inhibit the hydrolysis of maltose and starch by the glucoamylases (Table 5). The enzymes from liver and spleen were inhibited by turanose to a greater extent (approx. 70%) than the intestinal enzyme (approx. 28%). Turanose inhibition has been used as suggestive evidence for a lysosomal origin of certain enzymes (Lejeune et al. 1963). Tris was a more powerful inhibitor than pentaerythritol.

Sephadex G-200 chromatography of liver, spleen and intestinal glucoamylases. The partially purified enzyme preparations of liver and spleen behaved similarly during gel filtration on Sephadex G-200 columns and emerged after the void volume of the column. The behaviour of the intestinal enzyme, which emerged near the void volume, was quite different (Fig. 2).

Identity of the maltase fraction associated with glucoamylase activity in monkey intestine

(a) Heat-inactivation studies on intestinal glucoamylase. By heat-inactivation studies it has been shown (Swaminathan & Radhakrishnan, 1970) that three species of maltase are present in the monkey intestinal mucosa. The present studies with both particulate and solubilized fractions suggest that glucoamylase is associated with them maltase II, which accounts for about 25–30% of the total maltase activity (Fig. 3).

(b) DEAE-Sephadex chromatography. The supernatant fraction after bentonite treatment was used for this purpose. Initially it was found that maltase I activity could be substantially eliminated by heating at 55°C for 5min and that such a fraction with diminished maltase I activity gave good
Table 4. $K_m$ and $V_{max}$ values for glucoamylase in various monkey tissues

Standard assay conditions were employed, and the final purified enzymes were used. $V_{max}$ values are given as $\mu$mol of glucose formed/min per mg of protein.

<table>
<thead>
<tr>
<th>Source of enzyme</th>
<th>Substrate</th>
<th>Starch</th>
<th>Maltose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_m$ (mg/ml)</td>
<td>$V_{max}$</td>
<td>$K_m$ (mM)</td>
</tr>
<tr>
<td>Liver</td>
<td>8.3</td>
<td>3.1</td>
<td>19.0</td>
</tr>
<tr>
<td>Spleen</td>
<td>9.0</td>
<td>7.5</td>
<td>10.0</td>
</tr>
<tr>
<td>Intestine</td>
<td>1.6</td>
<td>12.2</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Table 5. Inhibition studies on glucoamylase in various monkey tissues

Standard assay conditions were employed; the activity was expressed with respect to controls without the inhibitors, the value of which was taken as 100.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Glucoamylase</th>
<th>Maltase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Spleen</td>
</tr>
<tr>
<td>Tris (0.1 M)</td>
<td>89</td>
<td>89</td>
</tr>
<tr>
<td>Pentaerythritol (0.1 M)</td>
<td>29</td>
<td>16</td>
</tr>
<tr>
<td>Turanose (0.03 M)</td>
<td>72</td>
<td>67</td>
</tr>
</tbody>
</table>

Fig. 2. Chromatography on Sephadex G-200. For the intestinal enzyme a papain-solubilized sample was used. For liver and spleen enzymes, concentrated DEAE-Sephadex fraction was used. The flow rate was 10 ml/h. Fractions (3 ml) were collected. For details see the text. •, Intestine; ■, spleen; ▲, liver.

separations of the three maltases by DEAE-Sephadex chromatography. Three peaks of maltase activity were obtained and the glucoamylase activity was clearly associated with maltase II. There was no detectable glucoamylase activity in the regions of maltase I or III activity (Fig. 4).

(c) Effect of metal ions and urea. By using the maltase II fraction isolated by DEAE-Sephadex chromatography, the effect of various concentrations of metal ions and urea on maltase and glucoamylase activities was studied. Ag$^+$ and Hg$^{2+}$ inhibited both the activities to the same extent at different concentrations of the metal ions. Thus at 0.01 mM the inhibition with Ag$^+$ was 87% and with Hg$^{2+}$ it was 20%. Similarly, it was found that the presence of various concentrations of urea in the incubation mixture caused a parallel inhibition of both the activities.

(d) Mixed-substrate inhibition studies. Mixed-substrate inhibition studies with starch and various concentrations of maltose showed that maltose and starch inhibited the hydrolysis of each other (Table 6).

Thus from the above studies it is clear that the glucoamylase activity of monkey intestine is associated with the maltase II fraction.

DISCUSSION

The results presented in the present paper suggest that the intestinal glucoamylase differs from the liver and spleen enzymes in several properties. The
intestinal enzyme has a higher pH optimum and shows a higher affinity towards maltose and starch compared with the liver and spleen enzymes. Its behaviour on Sephadex columns is also quite different from that of the other two enzymes.

Swaminathan & Radhakrishnan (1970) showed that monkey intestine contains three maltases as ascertained by heat-inactivation experiments. A similar result was obtained for pig (Dahlqvist, 1959) and human (Dahlqvist, 1962). The glucoamylase activity was not associated with maltase I, but whether it was associated with maltase II or maltase III or both has not been unequivocally demonstrated (Dahlqvist & Thomson, 1963; Eggermont & Hers, 1969). In the present study, glucoamylase activity of monkey intestine is associated with the maltase II fraction. The identical rates of heat-inactivation, mutual inhibition as shown by mixed-substrate incubation studies and the parallel inhibition by Ag²⁺, Hg²⁺ and urea, all show that maltose and starch are hydrolysed by the same enzyme. Melezitose, turanose and phenyl α-D-glucoside are also hydrolysed by this purified fraction from the intestine. However, the hydrolysis of these substrates could be distinguished from that of maltose by heat-inactivation studies. For example, heating the enzyme preparation at 60°C for 10 min resulted in the inactivation of over 95% of maltase and glucoamylase activities, whereas the activity

Table 6. Mixed-substrate incubation of glucoamylase preparation

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Glucose formed (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch (5mg)</td>
<td>16.4</td>
</tr>
<tr>
<td>Maltose (0.2mm)</td>
<td>13.6</td>
</tr>
<tr>
<td>Maltose (0.4mm)</td>
<td>23.4</td>
</tr>
<tr>
<td>Maltose (0.8mm)</td>
<td>37.2</td>
</tr>
<tr>
<td>Starch (5mg)+maltose</td>
<td>21.8</td>
</tr>
<tr>
<td>+ maltose (0.2mm)</td>
<td>25.0</td>
</tr>
<tr>
<td>+ maltose (0.4mm)</td>
<td>28.6</td>
</tr>
<tr>
<td>+ maltose (0.8mm)</td>
<td>39.8</td>
</tr>
</tbody>
</table>

Fig. 3. Stepwise heat-inactivation of intestinal maltase (○) and glucoamylase (●). The pH of the incubation mixture was 7.0 and the sodium phosphate concentration was 10mM. Protein concentration was 10mg/ml. Samples were taken as indicated at different times for assay of enzyme activity.

Fig. 4. Gradient-elution chromatography of monkey intestinal maltase (○) and glucoamylase (●) on a DEAE-Sephadex column. ----, Buffer concentration. For details see the text.
towards turanose, melezitose and phenyl-α-D-glucoside was decreased by 23, 10 and 22% respectively (B. Seetharam, N. Swaminathan & A. N. Radhakrishnan, unpublished work). Further work is needed to establish whether these are hydrolysed by different enzymes. The association of maltase activity with glucoamylase activity in liver and spleen is also seen from the nearly constant ratio of the rates of hydrolysis of maltose and starch during purification. This ratio was close to 2 in liver and was 1 in spleen. The maltase activity (m-units/mg of protein) of liver (4800) and spleen (4500) was of the same order but the glucoamylase activity (m-units/mg of protein) of liver (2816) was much lower than that of spleen (5000) and the observed difference in the ratios with the two tissues may therefore be due to the lower glucoamylase activity in liver. In the present studies the variation in the ratio during purification was much less marked than reported by Fujimori et al. (1968). With the intestine there is a marked change in the maltase/glucoamylase ratio during purification. Since glucoamylase activity in this tissue is associated with maltase II, which is one of the three maltases present, the change in the ratio is to be expected as a consequence of removal of the other two maltases during purification. The purified intestinal enzyme, giving a maltase/glucoamylase ratio of 1, thus resembles the spleen enzyme.

Although there is evidence suggesting that maltase activity was associated with glucoamylase in liver, kidney and intestine, Fujimori et al. (1968) have shown that these activities could be distinguished in the purified bovine spleen enzyme by heat-inactivation, pH optima, inhibition by turanose and the ratio of the two activities during purification. However, in the present study with preparations from monkey spleen the two activities were affected to the same extent by inhibitors such as turanose, tris and pentaerythritol. Also, the optimum pH for the hydrolysis of both substrates and the maltose/starch hydrolysis ratio was essentially the same.

Auricchio & Bruni (1967) found that the liver and kidney glucoamylases were retarded by Sephadex owing to an enzyme–substrate type of interaction. Although the results in the present study show a similar behaviour of the liver and spleen enzymes, the intestinal enzyme appears to be quite different. It has been suggested (Rosenfeld, 1964) that this retardation was due to the dextranase activity of the enzyme preparations and in support of this Bruni, Auricchio & Covelli (1969) have reported that the enzyme from bovine liver hydrolyses α-(1→6)-glucosidic linkages of dextran and isomaltose. In these studies the relative isomaltase activity was approx. 4% of the maltase activity. By using a partially purified enzyme from dog liver a similar result was observed by Torres & Olavarria (1964). In the present study the behaviour on Sephadex G-200 columns of the glucoamylase fractions from monkey liver and spleen was similar to earlier reports for a liver enzyme. On the other hand, the intestinal enzyme behaved differently even in the presence of high isomaltase activity in the fractions. However, it is to be noted that in the intestine, isomaltase activity is rather unstable during purification or when kept in the frozen state (Dahlqvist, 1960; Swaminathan & Radhakrishnan, 1970). Intestinal isomaltase probably exists as a ‘complex’ with invertase and the major maltase, as in the human (Dahlqvist & Telenius, 1969), in rabbit (Kolinska & Semenza, 1967) and in monkey (Swaminathan & Radhakrishnan, 1970). In the rat intestine, the dextranase activity was associated with isomaltase activity (Dahlqvist, 1963). In the present study, glucoamylase activity is not associated with the isomaltase-containing maltase fraction and it is therefore possible that, at least in the intestinal preparation, the presence of isomaltase may indicate contamination of the glucoamylase.

The glucoamylases of liver and spleen are presumably of lysosomal origin (Lejeune et al. 1963; Fujimori et al. 1968) whereas the intestinal enzyme is present in the brush-border region of the epithelial cell (Ruttloff et al. 1967). The inhibition by turanose and acid pH optimum would indicate a lysosomal origin of certain enzymes (Torres & Olavarria, 1964) and this is substantiated with liver and spleen enzymes. The intestinal enzyme is also inhibited by turanose though to a smaller extent in spite of a different cellular localization.

The importance of glucoamylase in the degradation of glycogen in addition to the phosphorolytic cleavage has been pointed out by Rosenfeld (1964). The intestinal enzyme may have a role similar to that of liver and spleen enzymes and with its favourable kinetic characteristics (low $K_m$ and high $V_{max}$) it may also be involved to a limited extent in the hydrolysis of dietary starch and glycogen.

The authors deeply appreciate the keen interest of Professor S. J. Baker in this work. The project was supported in part by U.S. Public Law 480 funds under Contract no. 0132901.

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