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SEPARATION AND CHARACTERIZATION OF SUCRASE–ISOMALTASE AND OF GLUCOAMYLASE OF RAT INTESTINE

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SUMMARY

1. Sucrase–isomaltase and glucoamylase (α-1,4-glucan glucohydrolase, EC 3.2.1.3) from the intestine of adult rats were separated from each other after solubilization by papain (EC 3.4.4.10), and chromatography on Sephadex G-200 and DEAE-cellulose. Both enzymes were obtained in a highly purified form.

2. The sucrase-1 component was found to arise artificially from the sucrase–isomaltase complex after ethanol precipitation of the papain-solubilized material.

3. Some properties (K_m, K_i, pH optimum, heat stability, activation by Na^+, immunochemical features) of these enzymes were investigated.

4. *p*-Nitrophenyl-α-glucoside and 6-bromo-2-naphthyl-α-glucoside were competitive inhibitors of sucrase and isomaltase activities of the sucrase–isomaltase complex and of glucoamylase and were hydrolyzed by both enzyme preparations.

INTRODUCTION

The brush border of intestinal epithelial cells of different species contains fundamentally three maltases. Two of them are associated with sucrase (sucrose glucohydrolase) and isomaltase (oligo-1,6-glucosidase, EC 3.2.1.10) activities, respectively, and have been isolated as a sucrase–isomaltase complex from rabbit[1] and human intestine[2,3]. The third, more heat-stable maltase with associated glucoamylase (α-1,4-glucan glucohydrolase, EC 3.2.1.3) activity occurs in the intestine of rat[4,5], man[6] and monkey[7]. A highly purified preparation of glucoamylase from young rats was recently obtained by Schlegel-Haueter *et al.*[8].

On sedimentation of Triton X-100-solubilized normal human intestinal mucosa in a density gradient[9], all three maltases sediment together. This observation led to the conclusion that the three maltases are naturally linked to each other in the brush border membrane. However, both on Sephadex[10,11] and during ion-exchange chromatography[12,13] the heat-stable maltase(s) appeared in a peak different from that containing sucrase and isomaltase activities.

This paper deals with the separation and purification of the sucrase–isomaltase complex and of glucoamylase from the intestine of adult rats. Comparison of the kinetic parameters of these enzymes towards both natural and artificial substrates is presented. A preliminary report on some data has appeared[14].

MATERIALS AND METHODS

Solubilization and purification procedure

The intestinal jejunum of 25 rats was rinsed in cold saline, the mucosa was scraped off and 30 g of the material were homogenized with 150 ml 0.005 M EDTA (pH 7.0), 0.5 M NaCl and 0.5 M KCl in a Waring blendor. This high ionic strength of the homogenization mixture was recommended earlier[1]. The homogenate was centrifuged at 60 000 × *g* for 60 min, the supernatant discarded and the sediment washed with 150 ml 0.1 M potassium phosphate buffer (pH 7.4) to diminish the contamination with pancreatic α-amylase (α-1,4-glucan 4-glucanohydrolase, EC 3.2.1.1). The sediment was incubated with 15 mg each of papain and cysteine–HCl in 50 ml 0.1 M potassium phosphate buffer (pH 7.4) for 60 min at 37 °C. After papain treatment, the 105 000 × *g* (60 min) supernatant was either precipitated with 2 vol. of ethanol at −15 °C and the sediment taken up in 10 ml of 0.01 M potassium phosphate (pH 7.4), or concentrated by negative pressure dialysis against 0.01 M potassium phosphate (pH 7.4). Both preparations were centrifuged again at 105 000 × *g* for 60 min to remove the precipitating components and applied to Sephadex G-200 columns of standard size, equilibrated with 0.01 M potassium phosphate buffer (pH 7.4).

The peaks of sucrase activity were further purified on DEAE-cellulose. Before application of the enzyme the cellulose columns were washed with 500 ml of 0.01 M potassium phosphate (pH 7.4). After adsorption of the enzyme on DEAE-cellulose, the columns were washed again with 0.01 M potassium phosphate buffer to bring the pH to 6.5. The chromatogram was then developed by applying a shallow Cl^- gradient. The peaks of sucrase and heat-stable maltase obtained were concentrated by negative pressure dialysis to a concentration of about 1–2 mg protein/ml and stored frozen before use.

Guinea-pig antisera to rat sucrase

Two guinea-pigs were immunized with sucrase preparations obtained from the rat small intestine, one animal with sucrase–isomaltase (S_2) and the other with sucrase devoid of isomaltase activity (S_1). The immunization was carried out using Freund's complete adjuvant. The immunization schedule was that recommended by Cummins *et al.*[2]. Enzyme solution (S_1 containing 1.5 mg protein and 17.1 sucrase units/ml or S_2 containing 1.25 mg protein and 13.95 sucrase units/ml) was emulsified at a 1:1 ratio with Freund's adjuvant. A total amount of 0.45 mg protein (S_1) or 0.5 mg protein (S_2) was injected intracutaneously and/or subcutaneously on several sites into the shaved back of the animal. Immunization was repeated on days 14 and 21 (S_1), and 12 and 19 (S_2), respectively. Blood was drawn by heart puncture on day 28 (S_1) or 26 (S_2). The antisera obtained were designated as 2/I for S_1 and 2/II for S_2. Precipitating antibodies were detected by the Ouchterlony test (double diffusion in agar gel) and by quantitative immunoprecipitation. A single precipitating line was observed

in agar gel[15] when the appropriate antigen and antiserum couple (S_1–2/I, or S_2–2/II) were employed. This precipitating line also retained the sucrase activity of the antigen, when stained by a sandwich technique of Lojda[16], with sucrose as substrate.

Titer of precipitating anti-sucrase antibodies

The titer of antibodies was estimated from exponential dilutions of the antiserum previously dialyzed against saline to remove endogenous glucose. 0.025 ml of the enzyme preparation (S_1 or S_2) was added to 0.1 ml of the antiserum (undiluted or diluted in a series to 1:16 with saline). Control samples contained the enzyme and dialyzed normal guinea-pig serum mixed together in the same way. The mixtures were incubated for 48 h at 4 °C and centrifuged. The supernatants were used for sucrase assay. Increasing the dilution of the antisera resulted in a reduction of the immunoprecipitating capacity so that higher amounts of the antigen (sucrase) appeared in the supernatant. The titer of antibodies was then expressed as the maximal amount of enzyme in units precipitated theoretically by 1 ml of the undiluted serum. This value was obtained as a difference between supernatant sucrase activity in controls (normal guinea-pig serum) and in samples with antisera at corresponding dilutions.

Analytical methods

Unless otherwise stated, disaccharidase activity was determined in 0.03 M lithium maleate buffer (pH 5.9) with 25 mM NaCl and a disaccharide concentration of 0.05 M, at 37 °C. Glucoamylase and dextranase (α-1,6-glucan 6-glucanohydrolase, EC 3.2.1.11) activities were measured with 12 mg starch and 45 mg dextran per ml. When hetero-α-glucosides served as substrates, 0.17 mM 6-bromo-2-naphthyl-α-glucoside and 11 mM *p*-nitrophenyl-α-glucoside were used. The reaction was stopped by 2 min boiling, and the glucose liberated was measured with the Tris–glucose oxidase–peroxidase reagent[17]. The 6-bromo-2-naphthyl-α-glucosidase activity was assayed by the method of Dahlqvist *et al.*[18] except that for substrate solubilization ethanol was used instead of methylglycol. The *p*-nitrophenol liberated was measured essentially by the procedure of Asp and Dahlqvist[19]. One unit of disaccharidase or hetero-α-glucosidase splits 1 μmole of substrate under the conditions used. One unit of glucoamylase or dextranase activity is defined as the amount of enzyme required to produce 1 μmole of glucose/min at 37 °C.

Protein was measured by the method of Lowry *et al.*[20] with human serum albumin as standard.

Electrophoresis on polyacrylamide-gel was performed at pH 8.2 in a gel gradient from 4.75 to 12.5%. The enzyme sample was applied with a slurry of Bio-Gel P-200 and run at 2–4 mA per tube. Protein bands were revealed with amido black.

Chemicals

Sucrose, isomaltose, maltose, 6-bromo-2-naphtyl-α-glucoside, *p*-nitrophenyl-α-glucoside, dextran (mol wt 60 000–90 000), glucose oxidase and peroxidase were purchased from Koch-Light Laboratories Colnbrook, England. Crystalline papain was obtained both from Mann Laboratories, New York and Koch-Light Laboratories. Sephadex G-200 was a product of Pharmacia A.B., Uppsala, Sweden; diethylaminoethyl cellulose DE22 of Balston, England. 6-Bromo-2-naphthol and naphthanil diazo blue B were obtained from Dajac Laboratories, Philadelphia, Pennsylvania;

TABLE I

EFFECT OF ETHANOL PRECIPITATION ON α-GLUCOSIDASE ACTIVITIES FROM THE RAT SMALL INTESTINE EXPRESSED IN TOTAL UNITS

	First sediment after washing	*Supernatant after papain solubilization*	*Sediment after ethanol treatment*
Maltase	380	308	156.5
Recovery (%)	100	81	41.2
Sucrase	92.7	74	63.4
Recovery (%)	100	79.8	68.3
Dextranase	74	58.7	20.3
Recovery (%)	100	79.4	27.4
Isomaltase	71.5	60.5	23
Recovery (%)	100	84.5	32.2

p-nitrophenol from the Corporation for Biochemical Research, Los Angeles, California. Serum albumin was obtained from the Institute of Vaccines and Sera, Prague, Czechoslovakia. All other materials were reagent-grade commercial preparations.

RESULTS AND DISCUSSION

Disaccharidase activities after and without ethanol precipitation

Ethanol precipitation of papain-solubilized α-glucosidases was used as a part of the purification procedure. Table I shows that ethanol precipitation brings about inactivation especially of dextranase and isomaltase.

No protection by potassium phosphate occurred, which is at variance with human disaccharidase activities[13] whose recovery after ethanol precipitation in the presence of potassium phosphate buffer was 85–100%.

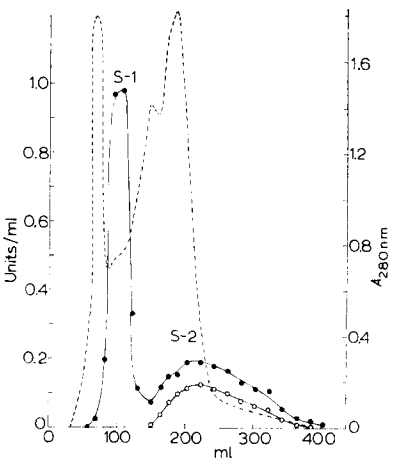
Sephadex G-200 chromatography

The behavior of rat sucrase on gel filtration chromatography was influenced by whether or not previous ethanol precipitation was performed.

(a) With previous ethanol precipitation. Rat jejunal preparation solubilized with papain, precipitated with ethanol and redissolved before chromatography yielded two sucrase peaks on the G-200 column, the first of which (sucrase-1) was eluted close to the void volume, the second (sucrase-2) being somewhat delayed (Fig. 1). The main difference between sucrase-1 and sucrase-2 is the association of dextranase activity with sucrase-2, whereas the peak of sucrase-1 contained no dextranase activity.

(b) Without previous ethanol precipitation. The Sephadex G-200 chromatography of the supernatant after papain treatment revealed one retarded broad peak of sucrase activity also containing dextranase (Fig. 2). In addition, a small sucrase activity peak substantially overlapping dextranase activity was seen a little ahead of the large sucrase peak; however, it accounted for only a very small part of the total sucrase activity.

Maltase activity is also given in Fig. 2. The first (void volume) maltase peak, obviously not belonging to the sucrase fraction, was partially separated from the second maltase peak associated with sucrase and dextranase.



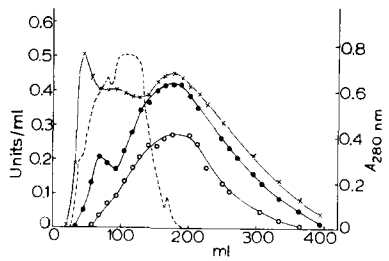


Fig. 1. Sephadex G-200 chromatography of the ethanol-precipitated material after solubilization by papain. The total volume of the column was approximately 250 ml. ●—●, sucrase; ○—○, dextranase; — — —, protein ($A_{280\,nm}$).

Fig. 2. Sephadex G-200 chromatography of papain-solubilized material. The total volume of the column was approximately 250 ml. ●—●, sucrase; ○—○, dextranase; ×—×, maltase; — — —, protein ($A_{280\,nm}$).

Two important points follow from these results.

(1) Ethanol precipitation altered the results of gel filtration chromatography of rat α-glucosidases so that, owing to considerable inactivation of dextranase, some sucrase without dextranase activity emerged shortly after the void volume; the second sucrase component carrying the preserved dextranase was retained by Sephadex owing to dextranase activity and subsequent adsorption to the stationary phase. This strongly indicates that sucrase-1 arose artificially from sucrase-2–isomaltase and supports the previous explanation of this artifact[21]. Dahlqvist and Telenius[13] reported a similar effect of ethanol on fractionation of human jejunal sucrase, apparently corresponding to that obtained by Semenza *et al.*[10]. There was no such separation of two sucrase fractions without ethanol treatment[13].

(2) Species differences ought to be discussed here. The sucrase–isomaltase complex isolated from rabbit intestine[1] seems to be fairly resistant to the action of ethanol. It was also considerably more retarded on Sephadex than rat (and human[2] and monkey[22]) sucrase and thus well separated from other intestinal proteins. On the other hand, the sucrase fraction of rat jejunum obtained either with or without previous ethanol precipitation could not be completely purified on Sephadex G-200 even when a longer column (100 cm × 2.5 cm) was used. Contamination with heat-stable maltase was found (see below). The last-mentioned enzyme was recently found to be retained by Sephadex[8] (possibly owing to its isomaltase activity) and therefore not effectively separated from sucrase–isomaltase in our procedure.

Gradient-elution chromatography on DEAE-cellulose performed with Sephadex G-200 fractions

The fractions (60–120 ml and 185–380 ml in Fig. 1) obtained from the Sephadex G-200 column were used for chromatography on DEAE-cellulose. The elution

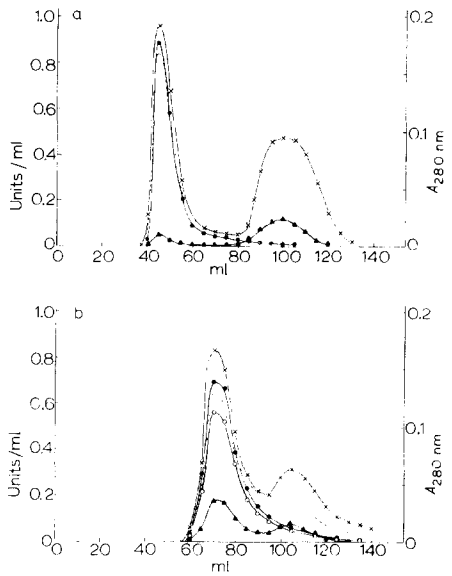


Fig. 3. (a) DEAE-cellulose chromatography of the sucrase-1 pool from a Sephadex column. ●—● sucrase; ×—×, maltase; ▲—▲, isomaltase; — — —, protein ($A_{280\ nm}$). (b) DEAE-cellulose chromatography of the sucrase-2 pool from a Sephadex column. ●—●, sucrase; ×—×, maltase ○—○, dextranase; ▲—▲, isomaltase; — — —, protein ($A_{280\ nm}$). For conditions see Materials and Methods.

chromatograms of sucrase-1 (Fig. 3a) and sucrase-2 (Fig. 3b) with chloride gradient resulted in separation of both sucrase-1 and sucrase-2 from a maltase activity peak also carrying isomaltase activity as indicated. For reasons which follow from other observations (see below), we shall call this fraction glucoamylase (heat-stable maltase).

While sucrase-1 only had traces of isomaltase activity, sucrase-2 was associated with a high amount of isomaltase.

These results may be compared with those of Dahlqvist[12] concerning separation of trypsin-solubilized rat intestinal α-glucosidases on TEAE-cellulose, performed after previous precipitation with ethanol. While gel filtration was not employed in his procedure, the gradient elution chromatography yielded two sucrase peaks eluted closely after each other, dextranase and isomaltase being a part of the second peak only, thus resembling our sucrase-1 and sucrase-2. The third peak with maltase and glucoamylase activities obviously resembled the one separated in our experiments from each of the two sucrases on DEAE-cellulose.

With human intestinal preparations the results of the ion-exchange chromatography were irrespective of whether or not[13] ethanol was previously applied. Sucrase and isomaltase activities were all recovered in the first large maltase peak; the second maltase peak was due to heat-stable maltase(s).

Criteria of purity and substrate specificity of sucrases

Pooled fractions, belonging to the DEAE-cellulose peak of sucrase-1 or sucrase-2–isomaltase, were concentrated by negative pressure dialysis or ultrafiltration. In

polyacrylamide-gel electrophoresis in a concentration gradient of gel, the sucrase-2–isomaltase complex was homogeneous, giving a single band.

Two closely accompanying bands, possibly due to contaminant or dissociation in Tris-buffer during electrophoresis (see comment in Schlegel-Haueter *et al.*[8]), were obtained with the preparation of sucrase-1. The guinea-pig antisera induced with sucrase-1, reacted in immunoelectrophoresis with sucrase-1 by also developing two precipitation lines (unpublished results).

As mentioned above, sucrase-1 is believed to arise artificially from the sucrase–isomaltase complex as a result of partial inactivation of isomaltase and dextranase by ethanol. Sucrase-1 should be rather called "altered sucrase-2–isomaltase" as it still has a weak isomaltase activity. The data available do not permit any speculation as to whether ethanol exerts its action on the link between sucrase and isomaltase.

Recent data of Cogoli and Semenza[23] obtained under different conditions show that it is possible to separate the isomaltase moiety from the sucrase–isomaltase complex of rabbit.

The non-identity of intestinal sucrase and isomaltase (both carrying maltase activity) was reported in rabbit[1], man[24] and monkey[25]. Evidence for the two active sites of the sucrase-2–isomaltase complex of rat intestine is based on the observation that there is no mutual inhibition between sucrose and isomaltose, and between sucrose and dextran. On the other hand, mutual inhibition between sucrose and maltose, maltose and isomaltose, and isomaltose and dextran was found (see Table II).

TABLE II

MIXED-SUBSTRATE INCUBATION WITH SUCRASE-2–ISOMALTASE PREPARATION

The incubation mixture (0.1 ml) contained one or two substrates (10 mM each, dextran 45 mg/ml) The incubation with enzyme was stopped after 15 min, and the liberated glucose was determined

Substrate	*Glucose formed (μg)*	
	Observed	*Calculated from individual substrates*
Sucrose	3.82	
Maltose	14.70	
Isomaltose	4.17	
Dextran	10.40	
Sucrose + isomaltose	8.13	7.99
Sucrose + dextran	13.86	14.22
Sucrose + maltose	16.18	18.52
Isomaltose + maltose	7.74	18.87
Isomaltose + dextran	8.66	14.57
Maltose + dextran	19.55	25.10

Table III shows specific activities of hydrolysis of some substrates by sucrase-1 and sucrase-2–isomaltase. Previous precipitation by ethanol does not change the final individual activities of the purified sucrase-2–isomaltase. Sucrase–isomaltase also splits 6-bromo-2-naphthyl-α-glucoside and *p*-nitrophenyl-α-glucoside, the latter being a relatively fine substrate. The question arose which of the two binding sites (for sucrose or isomaltose) is able to split the artificial substrate. Our results show that

TABLE III

SPECIFIC ACTIVITIES EXPRESSED AS INTERNATIONAL UNITS PER mg PROTEIN OF THE PURIFIED SUCRASE-I AND SUCRASE-2–ISOMALTASE

Hetero-α-glucosides were tested only with sucrase-2-isomaltase prepared without previous ethanol precipitation.

Substrate	*Sucrase-1*	*Sucrase-2–isomaltase after ethanol precipitation*	*Sucrase-2–isomaltase without ethanol precipitation*
Sucrose	9.6	10.8	11.8
Maltose	10.6	15.7	15.5
Isomaltose	0.3	3.9	3.3
Dextran	0	8.5	7.9
p-Nitrophenyl-α-glucoside	—	—	2.8
6-Bromo-2-naphthyl-α-glucoside	—	—	0.12

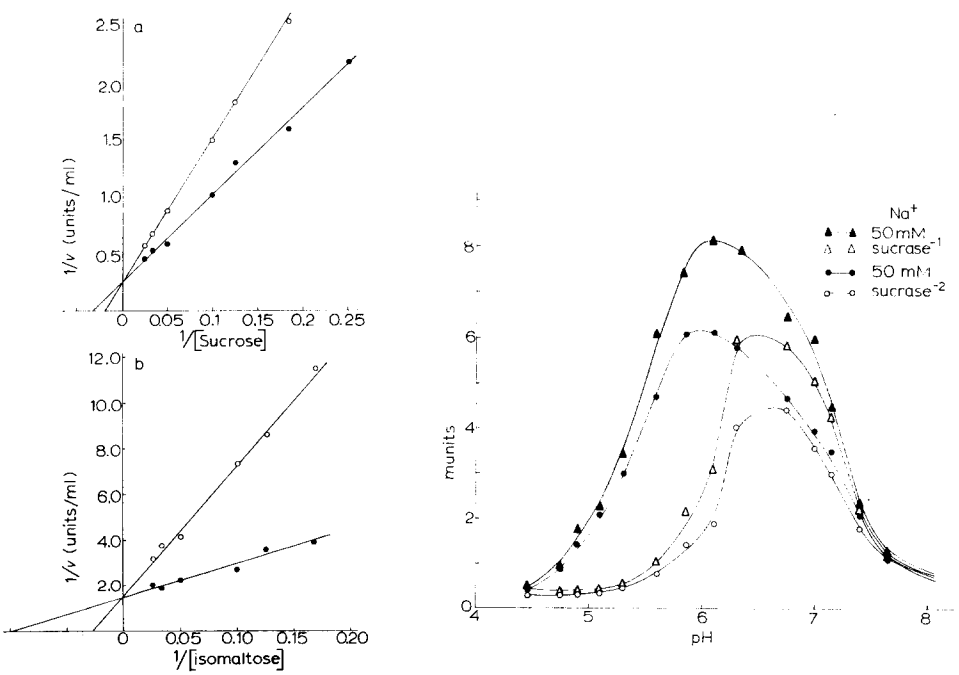


Fig. 4. Purified sucrase–isomaltase incubated with substrate in 0.02 M lithium maleate buffer (pH 5.9) and 16.5 mM NaCl. (a) Lineweaver–Burk plot of sucrase activity without and with 4.8 mM *p*-nitrophenyl-α-glucoside (○—○), control (●—●). (b) Lineweaver–Burk plot of isomaltase activity without and with 4.8 mM *p*-nitrophenyl-α-glucoside (○—○); control (●—●). Substrate concentration in mM.

Fig. 5. pH–activity curves for sucrase-1 (△, ▲) and sucrase-2 (○, ●). Buffers at constant Li^+ (25 mM): acetate (pH 4.4–4.9), maleate (pH 5.1–6.8), phosphate (pH 7.1–7.7). Curves without Na^+ (△, ○) and with 50 mM NaCl (▲, ●) are shown.

both can. *p*-Nitrophenyl-α-glucoside inhibits both sucrase and isomaltase in a competitive way (Fig. 4a and 4b). The same mode of inhibition was obtained with 6-bromo-2-naphthyl-α-glucoside; the K_i values are reported in Table IV. Taken together, the relative affinities of substrates and the K_i values indicate that the isomaltose binding site has a higher affinity for artificial substrates than does the sucrose binding site.

Competitive inhibition of rat maltase, which resembles that belonging to our sucrase–isomaltase complex, by 6-bromo-2-naphthyl-α-glucoside was previously reported by Dahlqvist *et al.*[26].

TABLE IV

V AND K_m VALUES FOR SUCRASE–ISOMALTASE AND GLUCOAMYLASE

Inhibition (K_i) by hetero-α-glucosides: 6-bromo-2-naphthyl-α-glucoside (BNG) and *p*-nitrophenyl-α-glucoside (PNPG) were used at concentrations of 0.16–0.26 mM and 4.4–5.0 mM, respectively. Incubation mixture contained 16.3 mM Na^+.

Enzyme	*Substrate*	*V (units/mg protein)*	K_m *(mM)*	K_i *(mM)*	
				PNPG	*BNG*
Sucrase–isomaltase	Sucrose	17.5	30.8	5.58	0.68
	Isomaltose	3.03	11.5	1.96	0.29
	Maltose	19.8	8.2*		
	PNPG	3.3	1.34*		
	BNG	0.42	0.12		
Glucoamylase	Maltose	29.5	1.24	0.77	0.11
	PNPG	0.83	3.58		
	BNG	0.44	0.18		

* See note added in proof p. 246.

Comparison of some properties of sucrase-1 and sucrase-2

Using the purified preparations of sucrase-1 and sucrase-2, the following common characteristics were obtained.

pH dependence. pH–activity curves showed the identity of sucrase-1 and sucrase -2 in this respect. Fig. 5 indicates the pH optimum, 6.7, of both sucrase-1 and sucrase -2 in the absence of Na^+. A marked activation and shift of the pH optimum to 5.9 is seen in the presence of 50 mM Na^+. This behavior is in agreement with that reported by Semenza[27] for hamster sucrase. The pH activity curves of rabbit and human sucrases, however, do not vary with Na^+ concentration[1,28,10].

Half-saturation constant. As obtained by a Lineweaver–Burk plot, the apparent K_m values of sucrase-1 and sucrase-2 in the absence of Na^+ are 47.5 and 42 mM, respectively. 40 mM Na^+ decreases the apparent K_m for sucrose. This applies to both sucrase-1 and sucrase-2, the K_m values found being 18,5 and 20 mM, respectively. This observation is in accordance with data on unsolubilized rat and hamster sucrases[29] and at variance with those on rabbit[1] and human[30] enzymes, in which Na^+ activation concerns maximal velocity.

Heat inactivation. At pH 5.9 and 50 °C, both sucrase-1 and sucrase-2 are inactivated, the former slightly faster than the latter (Fig. 6). Decrease of both the maltase and sucrase activity of sucrase-1 preparation follows first order kinetics. The inactivation of maltase associated with the sucrase-2–isomaltase complex, however, makes it probable that it consists of two components, one of which is inactivated at

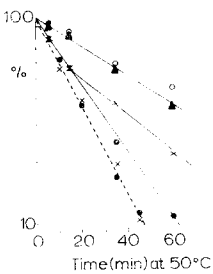


Fig. 6. Inactivation of sucrase-2–isomaltase and sucrase-1 at 50 °C and pH 5.9. Incubation with substrate at 37 °C for 15 min. Substrate concentration as indicated in Materials and Methods. — · — · —, sucrase-2–isomaltase; — — —, sucrase-1. Activities: ●, sucrase; ×, maltase; ○, dextranase; ▲, isomaltase.

the same rate as sucrase and the other as isomaltase (or dextranase). It is important to note that two sucrase fractions of human intestine[10] have a similar heat sensitivity, identical pH optima and the same degree of activation by Na^+.

Immunological cross-reaction of the two sucrase preparations S_1 and S_2. As shown previously[15], there was a positive cross-reaction in the Ouchterlony test in agar gel with S_1 and S_2 as antigens and the antisera 2/I and 2/II. A quantitative proof for this mutual cross-reaction is demonstrated in Table V.

TABLE V

IMMUNOLOGICAL CROSS-REACTION OF SUCRASE-1 AND SUCRASE-2

	Titer of precipitating antibodies per ml antiserum	
	Units of sucrase-1	*Units of sucrase-2*
Antiserum to S_1 (2/I)	11.32	17.98
Antiserum to S_2 (2/II)	5.13	6.62

Properties of glucoamylase (heat-stable maltase)

As the second maltase fraction from DEAE-cellulose chromatography (Figs 3a and 3b) was still slightly contaminated by tailing of the sucrase fraction, it was dialyzed against 0.01 M potassium phosphate buffer and rechromatographed again on DEAE-cellulose. Some traces of sucrase activity can be removed from this preparation by incubation for 30 min at 50 °C. This maltase preparation is essentially homogeneous on polyacrylamide gel electrophoresis. It has a high specific activity for maltose (42.7 units/mg P), and it splits starch (28.4 units/mg P), isomaltose (7.6 units/mg P) and the artificial substrates 6-bromo-2-naphthyl-α-glucoside (0.074 units/mg P) and *p*-nitrophenyl-α-glucoside (0.72 units/mg P). It does not split dextran. Other important substrates, such as oligosaccharides[5], were not studied here.

In its high specific activity for maltose this preparation resembles the one purified recently under different conditions by Schlegel-Haueter *et al.*[8] from 10–15-day-old rats. The activity ratio maltase/glucoamylase is similar to that calculated from the data of Alpers and Solin[5].

Maltase as well as glucoamylase activity was found to be stable at 50 °C and pH 5.9 during 60 min incubation. Both isomaltase and *p*-nitrophenyl-α-glucosidase activities decreased only by some 8% under the same conditions, indicating that these activities cannot belong to the relatively heat-labile sucrase–isomaltase complex. Moreover, competitive inhibition of maltose hydrolysis by *p*-nitrophenyl-α-glucoside (Fig. 7) lends support to the view that both substrates mentioned are hydrolyzed by the same enzyme.

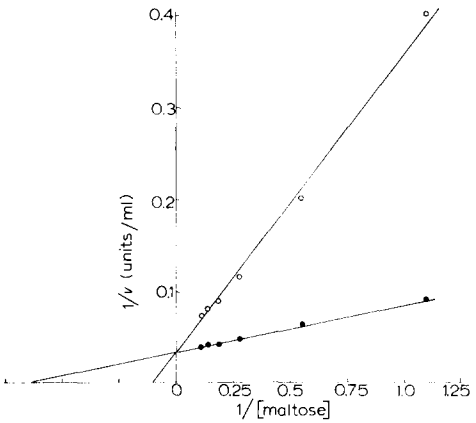


Fig. 7. Purified glucoamylase. Lineweaver–Burk plot of maltase activity without and with 4.4 mM *p*-nitrophenyl-α-glucoside (○—○). Lithium maleate buffer (pH 5.9) and 16 mM NaCl. Substrate concentration in mM. ●—●, control.

Unlike the sucrase (as well as maltase and isomaltase) activities of the sucrase–isomaltase complex, neither maltase nor isomaltase of the heat-stable enzyme preparation were activated to any appreciable extent by 50 mM Na^+, an activation by some 6–8% for both maltase and isomaltase having been found.

Our results on heat stability of isomaltase are in accord with the heat inactivation experiments of Dahlqvist and Telenius[13] showing that the heat-stable maltase of human intestine also has an isomaltase activity.

Our kinetic experiments fit well with those of Dahlqvist *et al.*[26], who found 6-bromo-2-naphthyl-α-glucoside to be a competitive inhibitor of the rat heat-stable maltase.

Lack of activation by Na^+ was reported by Semenza *et al.*[10] for the heat-stable maltase of human intestine.

In Table IV some kinetic parameters of the heat-stable maltase preparation are given. The reason for the discrepancy between the K_m value for *p*-nitrophenyl-α-glucoside and its K_i for inhibition of maltase is not known. The presence of more than one binding site for substrates which may affect each other cannot be excluded at present.

It is of interest in this connection that, according to Eggermont and Hers[9], the human glucoamylase corresponds at least partially to the heat-stable maltases II and III, recognized by Dahlqvist[31] on the basis of their slightly different heat sensitivity and by Semenza *et al.*[10] according to their appearance on Sephadex G-200

(void volume Fractions 1 and 2). In monkey[7] glucoamylase belongs to the heat-stable maltase II, whereas no glucoamylase was present in a separated small fraction of the heat-stable maltase III.

Summing up, combination of Sephadex and ion-exchange chromatography provided a successful separation and purification of the sucrase–isomaltase complex and of glucoamylase from the small intestine of adult rats. The specific activities of the sucrase–isomaltase complex compare well with those obtained in other species. Similarly, the degree of purification of our preparation of glucoamylase from adult rats compares favorably with that obtained from infant rats[8]. Infant rats represent, of course, better material for isolation of glucoamylase as they are essentially devoid of sucrase and isomaltase activities. However, for a comparison of the individual properties of multiple α-glucosidases in the intestinal mucosa, adult rats, providing a sufficient yield of all these enzymes, were chosen as experimental animals in the present work.

The glucoamylase fraction of infant rats must be separated from the contaminating lactase which is very active at this stage of development. These last-mentioned enzymes were successfully separated from each other on Sepharose[8]. On the other hand, α-glucosidases are inseparable on a Sepharose column: they were all (sucrase, isomaltase, glucoamylase) obtained by Forstner[32] in a single peak.

Immunochemical studies using the homogeneous fractions of intestinal α-glucosidases with emphasis on their molecular properties and cellular localization are now in progress.

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Our recent results using the Hofstee plot yield two K_m values for each of the substrates, 7.2 and 13.5 mM for maltose, and 1.5 and 3.1 mM for *p*-nitrophenyl-α-glucoside. The existence of two K_m values (though not differing from each other appreciably) supports the notion of substrate hydrolysis at two active sites on the sucrase–isomaltase complex.

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