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STUDIES ON INTESTINAL SUCRASE* AND ON INTESTINAL SUGAR TRANSPORT. V**. ISOLATION AND PROPERTIES OF SUCRASE–ISOMALTASE FROM RABBIT SMALL INTESTINE

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SUMMARY

1. The sucrase–isomaltase complex has been isolated from rabbit small intestine. The procedure involves urea extraction of the whole intact small intestine, papain solubilization, and chromatography on Sephadex G-200 and polyacrylamide-gel (Bio-Gel P-300).

2. The chromatography on Sephadex G-200 is apparently based on an interaction of the substrate–enzyme type.

3. Sucrase–isomaltase, as isolated here, carries at least two substrate sites: one splitting sucrose and maltose, the other splitting isomaltose, palatinose and probably maltose also.

4. Na^+ is a non-essential activator. It increases the maximum velocity by some 20–30%, with minor or no change of the apparent K_m for sucrose.

5. Tris is a strong fully competitive inhibitor.

6. The inhibition by some metals, and the lack of inhibition by other metals or by *p*-chloromercuribenzoate, indicate that SH groups are not required for full enzyme activity, whereas an imidazole group may be required.

INTRODUCTION

Sucrase (sucrose glucohydrolase) has been purified from the intestinal mucosa of the hog[2], the rabbit[3] and the rat[4]. Isomaltase (oligo-1,6-glucosidase, EC 3.2.1.10) has been partially purified from the ox[5] and the hog[6,7]. The sucrase–isomaltase complex has been partially purified from the rabbit[8], the rat[9] and the human intestinal mucosa[10]. Recently, particles with sucrase activity have been isolated from hamster[11] and from rabbit[12] small intestine.

* The trivial name 'invertase' is well established for β-fructofuranosidase (EC 3.2.1.26). Inasmuch as the small-intestinal enzyme is a glucosidohydrolase, as indicated by its substrate specificity, it seems more appropriate to designate it with another name.

** IV: ref. 1.

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In the present paper we report what we believe to be the first procedure for the isolation of intestinal sucrase–isomaltase. Also included are some kinetic data, particularly on the Na^+ activation of sucrase. The macromolecular properties and the chemical composition of sucrase–isomaltase will be the subject of a paper to follow*. A preliminary report has already appeared[13].

MATERIALS AND METHODS

Unless otherwise stated, sucrase and isomaltase activities were determined at pH 6.7 in 0.03 M sodium maleate buffer, at a substrate concentration of 0.050 M and at 37°. Incubation was stopped by boiling the sample or an aliquot thereof for 2 min. The liberated glucose was measured with the Tris–glucose oxidase–peroxidase reagent[14,15]. One unit of enzyme splits one μmole of substrate per min under the conditions of the assay.

Protein was measured by either the biuret[16] or LOWRY's[17] procedure.

Starch-gel electrophoresis was performed with starch obtained from the Connaught Medical Research Laboratories, Toronto, in the Tris, citrate (pH 8.65), borate discontinuous buffer system[18] for 3 h, at 4.5 V/cm. Polyacrylamide electrophoresis was performed at pH 9.5 using the upper and lower gels of the standard procedure suggested by CANALCO, Bethesda, Md. (see also ORNSTEIN AND DAVIS[19]). The sample was applied together with a slurry of beads of Bio-Gel P-20 polyacrylamide, and run for 2 to 3 h at 13 V/cm. Electrophoresis on cellulose acetate[20] was carried out in 0.06 M sodium veronal buffer (pH 8.6) containing 0.7 ml of mercaptoethanol per l, for 90 min, at 18 V/cm. The protein zones were revealed with Amidoblack.

Preparative chromatography on Sephadex G-200 was carried out in a glass tube from Pharmacia, Uppsala. The sample and the developer were fed from the bottom of the column with a "Perpex" peristaltic pump (W. Meyer, Luzern). All other column chromatographies were carried out with a flow from the top to the bottom.

Diluted sucrase solutions were concentrated, if necessary, by ultrafiltration or by dialysis against dry powdered sucrose[8,21].

Isomaltose and palatinose were generously given by Prof. WEIDENHAGEN (Neuoffstein, Pfalz, W. Germany). Valinomycin was generously given by Prof. H. BROCKMANN, (Göttingen, W. Germany). Crystalline papain[22] (EC 3.4.4.10) was obtained from Mann Laboratories, New York; Sephadex G-200 and dextran (mol. wt. approx. 2 000 000) from Pharmacia A.B., Uppsala; polyacrylamide beads P-20 and P-300 from Bio-Rad, Richmond, Calif. All other materials were reagent-grade commercial preparations.

The isolation procedure

The procedure finally adopted will be given first and comments on the individual steps will be included in the following section.

For each preparation 3 rabbits of either sex were used. The animals were killed by a blow across the neck, and the jejunum was excised. Unless otherwise stated, all

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subsequent steps were carried out in a cold room at approx. +4°. The excised jejunum was washed with cold saline, cut into 20-cm pieces, everted, and thoroughly washed several times with saline. Samples from each piece of intestine were pooled and used as 'starting material' for the determinations of protein and enzyme activities.

Step 1: Urea extraction. Approx. 150 g of everted intestine were shaken (130 strokes/min) for 15 min with 300 ml of 0.15 M NaCl + 0.01 M KCl. The intestine was then transferred to 300 ml of 2 M urea containing EDTA (0.005 M) and KCl (0.05 M) (pH 7.0), and again shaken for 15 min. The intestine was extracted twice more, each time with a fresh urea solution, and discarded.

Step 2: Homogenization at high ionic strength. Solid NaCl and KCl were added to the combined urea extracts to give a final concn. of 0.5 M each. This mixture was homogenized for 1 min at the top speed of a Waring blendor, and centrifuged at 60 000 × *g* for 75 min. The supernatant was discarded.

Step 3: Papain solubilization[23]. The sediment was thoroughly dispersed in 40 ml water, and the protein content was determined (biuret). Usually the proteins amounted to about 2 g. Enough potassium phosphate buffer (pH 7.5) was added to reach a concentration of 0.05 M. Papain and cysteine HCl were added (at least 0.1 mg of each per mg sample protein) and the mixture was incubated for 30 min at 37°. After centrifugation at 105 000 × *g* for 60 min the sediment was discarded.

Step 4: Alcohol precipitation. The supernatant was precipitated with 2 vol. of ethanol at about −20°. The sediment was taken up in 5 to 8 ml of 0.01 M potassium phosphate buffer (pH 7.5) and the solution was stored in the cold for at least 24 h and then centrifuged at 105 000 × *g* for 60 min.

Step 5: Sephadex G-200 chromatography. The supernatant was applied to the bottom of a column of Sephadex G-200 (2.6 cm × 95 cm) which had been equilibrated with 0.01 M potassium phosphate buffer (pH 7.5). The column was developed upwards with this buffer at a constant flow rate of about 4 ml/h. Fractions were collected every hour (Fig. 1).

Step 6: Polyacrylamide chromatography. The major peak of sucrase activity (for example, from ml 530 to ml 850 in Fig. 1) was concentrated to a volume of approx. 20 ml and applied to a column (3 cm × 22 cm) of polyacrylamide beads P-300 which had been equilibrated with the same potassium phosphate buffer. This column was also developed with this buffer (10 ml/h). Sucrase emerged immediately after the void volume.

Comments on the procedure

Potassium is known to stabilize most human intestinal disaccharidases[23]. Therefore, potassium was included in most solutions used throughout the preparation. Other unusual features of the isolation procedure are related to the fact that sucrase and isomaltase are components of, or are bound to, the membrane of the brush borders[24].

Step 1. Rabbit intestine is rich in mucus which must be eliminated as completely as possible by washing with saline.

Urea and a number of other substances have been reported to disrupt the brush borders of hamster small intestine into several fractions without solubilizing sucrase[24]. A. EICHHOLZ (personal communication, 1965) has further obtained a reasonably effective extraction of alkaline phosphatase from intact hamster intestine with 1 M Tris–HCl. In preliminary experiments we observed that either 1 M Tris–HCl, or 1 M urea (+ EDTA and KCl), both at pH 7.2, can extract most of the

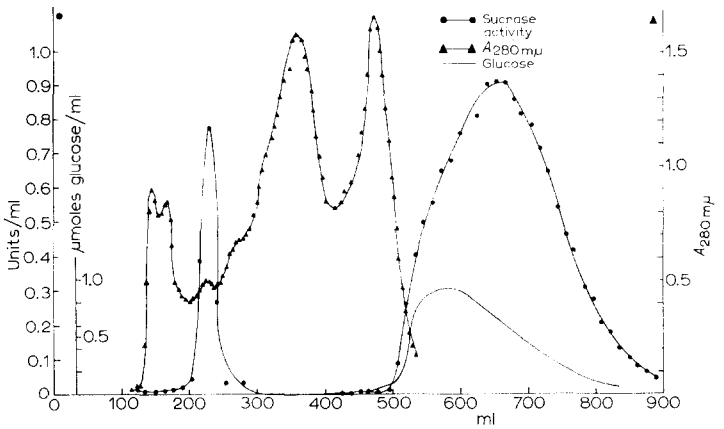

Fig. 1. Sephadex G-200 chromatography of papain-solubilized and ethanol-precipitated material. The total vol. of the column was approx. 500 ml. For conditions, see text.

sucrase from rabbit jejunum. We preferred urea to Tris, because the latter is a powerful competitive inhibitor of sucrase (see below), and a concentration of 2 M urea, instead of 1 M, because it was found to be more effective.

Urea extraction proved to be both more effective and less time-consuming than the usual procedure of mucosal scraping.

Step 2. When urea is applied to the intact intestine, as described above, whole cells, as well as brush border fragments, pass into the extract. Therefore, when this extract or its sediment is later subjected to papain solubilization, a solution is eventually obtained which is too viscous to be applied to the Sephadex column. Hyaluronidase (EC 4.2.99.1) was not found to reduce this viscosity, whereas pancreatic deoxyribonuclease (EC 3.1.4.5) worked effectively. Therefore, in order to dissociate the nucleohistone (permitting elimination of DNA in the subsequent supernatant), the urea extract was homogenized at high ionic strength.

Step 3. Solubilization of rabbit intestinal sucrase and isomaltase has been attempted in a number of ways. Trypsin (EC 3.4.4.4), pancreatic lipase (EC 3.1.1.3), snake venom[25], acetone and butanol[8] are reported to be scarcely effective. Deoxycholate[25] brings about an unstable solubilization. Sonication[25,8] solubilizes irreversibly at least 30–40% of sucrase activity. We have used papain digestion which we had previously found to be effective in solubilizing human disaccharidases[23]. The yield (Table I) is reasonably satisfactory.

TABLE I

SUMMARY OF A PREPARATION OF SUCRASE–ISOMALTASE FROM RABBIT SMALL INTESTINE

The units refer to the sucrase activity.

Fraction	*Total units*	*Total proteins (mg)*	*Specific activity*	*Recovery*
Starting material	2400	18 500	0.13	100
After urea extraction	700	1 891	0.37	29.2
After papain solubilization	565	828	0.67	23.4
Redissolved after ethanol precipitation	490	155	3.2	20.5
After Sephadex G-200 (major peak)	286	33	8.7	12.3
After P-300	218	15	14.5	9.1

Step 4. In addition to concentrating the sample for Sephadex chromatography, alcohol precipitation eliminates significant amounts of contaminating protein. After ethanol fractionation, it is necessary to store the sample in the cold for at least 24 h, to permit removal of precipitating components which might otherwise precipitate during the chromatography.

Step 5. Sephadex G-200 chromatography has proved valuable in separating human intestinal disaccharidases[10]. Essentially the same conditions were applied here for rabbit sucrase–isomaltase (Fig. 1).

Sucrase–isomaltase is retained by Sephadex G-200 by mechanisms other than simple gel filtration. In fact: (a) The K_d on Sephadex G-200 (approx. 1.4 in the experiment of Fig. 1) indicates an 'adsorption' on to the stationary phase. This is the more significant, as the mol. wt. of isolated sucrase–isomaltase (211 000)* would

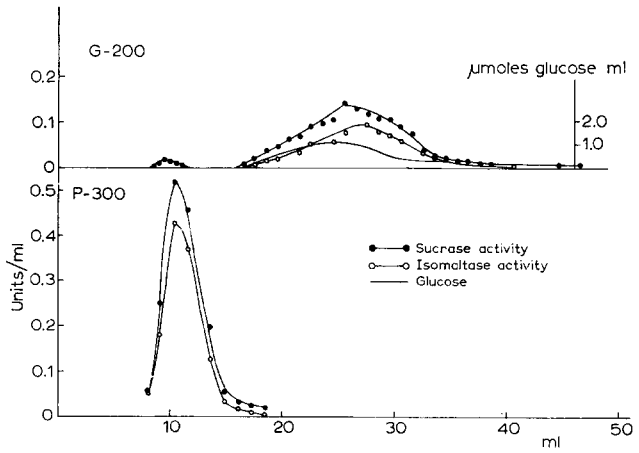

Fig. 2. Comparison between the chromatographic behaviour of isolated sucrase–isomaltase on Sephadex G-200 and on polyacrylamide gel P-300. In parallel experiments 2.2 sucrase units in 1.5 ml were applied to 1.1 cm × 21 cm columns. The columns were developed at a flow rate of 1.5 ml/h.

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correspond to a $K_d < 0.1$. Similar disagreement between mol. wt. and K_d on Sephadex G-200 has been observed with human sucrase–isomaltase (sucrase-2–isomaltase), which has a K_d of about 0.7 (ref. 10) and a mol. wt. of 202 000 as estimated by mannitol gradient centrifugation (E. EGGERMONT, unpublished results, 1964). (b) In parallel identical experiments on polyacrylamide gel (P-300) no such anomalous behaviour of sucrase–isomaltase was observed (Fig. 2) and, therefore, association–dissociation phenomena can be excluded as being responsible for the behaviour on

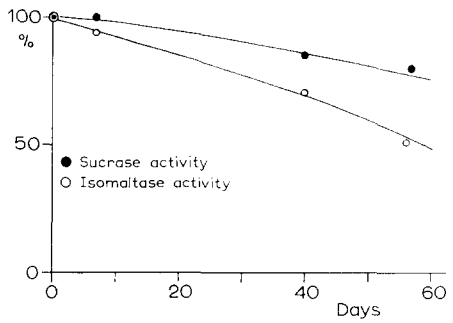

Fig. 3. Decline of sucrase and isomaltase activities during storage of sucrase–isomaltase in water at +4°.

Sephadex. (c) Ageing affects isomaltase activity more than sucrase activity (Fig. 3). If such an aged preparation is then run through Sephadex G-200, two peaks are produced (Fig. 4): one, emerging shortly after the void volume and having sucrase and maltase activities only; and a second peak, retained by Sephadex as much as in the original preparation, having sucrase, maltase and isomaltase activities. (d) Sucrase–isomaltase attacks Sephadex with liberation of free glucose (Figs. 1 and 2).

Apparently, therefore, sucrase–isomaltase is retained by Sephadex G-200

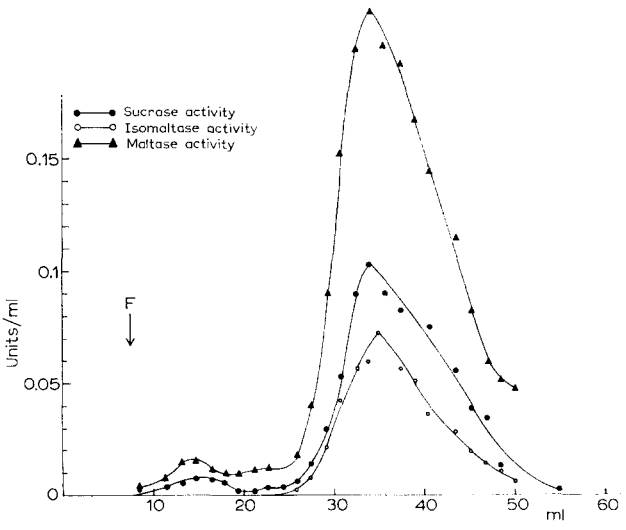

Fig. 4. Sephadex G-200 chromatography of an aged preparation (8 months at +4°). F, front.

owing to a substrate–enzyme interaction mainly involving its isomaltase site. (For further details see ref. 26.) Amylases are also known to be retained by Sephadex to a greater degree than one would expect from their mol. wt. (*e.g.*, refs. 27 and 28). Curiously, pullulanase, an enzyme specifically hydrolyzing some 1,6-α-glucosidic bonds, does not seem to interact with Sephadex G-200 in the same way[29] (see also NOTE ADDED IN PROOF).

The change in chromatographic behaviour on Sephadex G-200 following inactivation of the isomaltase site (Fig. 4) raises the question whether human intestinal sucrase-1, which has a K_d of about 0.23 and is not accompanied by isomaltase activity[10], might artificially arise from sucrase-2–isomaltase during papain digestion or autolysis by inactivation or separation of the isomaltase moiety. The mol. wt. reported by E. EGGERMONT (unpublished results, 1964) for sucrase-1 (138 000) is compatible with this possibility. Thus, although 2 sucrases are found in human intestinal mucosa after solubilization by at least three methods (autolysis[10], papain digestion[10], freezing and thawing[30]), the possibility still exists that they may both arise from one and the same hyphezyme (see below).

Criteria of purity

The final preparation obtained had a specific activity of about 14.5 sucrase units per mg protein (LOWRY). With a mol. wt. of approx. 211 000 it has, therefore,

TABLE II

MUTUAL EFFECT OF SUCROSE, ISOMALTOSE, PALATINOSE, MALTOSE, AND DEXTRAN ON THEIR HYDROLYSIS BY SUCRASE–ISOMALTASE

Each incubation mixture (0.5 ml) contained: one or two substrates (unless stated otherwise 5 μmoles each), sodium maleate buffer (pH 6.5; 7.5 μmoles), and the enzyme. After incubation at 37° for 15 min (in Expt. a) or 30 min (in Expt. b) the incubation was stopped by boiling the sample and the liberated glucose was determined.

Expt.	*Substrate*	*mμmoles glucose liberated*	*Calculated from the individual substrates*	*Inhibition found (%)*
a	Sucrose alone	31.8		
	Isomaltose alone	47		
	Maltose alone	214		
	Sucrose + isomaltose	85.7	78.8	—
	Isomaltose + maltose	228	261	12.6
	Maltose + sucrose	182	245.8	26
b	Isomaltose alone	87.8		
	Palatinose alone	55.0		
	Maltose* alone	85.5		
	Dextran** alone	7.85		
	Isomaltose + palatinose	80.6	142.8	43.5
	Isomaltose + maltose*	153.0	173.3	11.5
	Isomaltose + dextran**	88.4	95.65	7.6
	Palatinose + dextran**	21.1	62.85	66.5

* 0.5 μmole/0.5 ml incubation mixture.

** Mol. wt. approx. 2 000 000. Concentration corresponding to about 3 μmoles of glucose residues per 0.5 ml incubation mixture.

a turnover number of approx. 3000. The ratios sucrase:isomaltase:maltase are approx. 1:0.75:2.1.

The preparations with a sucrase specific activity of 14.5 were homogeneous according to the following criteria: (a) DEAE-cellulose chromatography developed with a shallow Cl^- gradient yielded a single peak having essentially the same specific activity in the individual fractions[13]; (b) one single band was obtained at different pH values in starch-gel, in polyacrylamide and cellulose acetate electrophoresis (see under MATERIALS AND METHODS); and (c) one single symmetrical peak was obtained in the ultracentrifuge under various conditions*,[13].

Evidence for two active sites in sucrase–isomaltase complex

Although the enzyme preparation obtained was homogeneous according to the usual criteria (see above), sucrose and isomaltose are split by 2 different active sites: (a) Ageing affects isomaltase activity more than sucrase activity (Fig. 3). (b) Sephadex G-200 chromatography of aged preparations separates 2 types of sucrase: one with and one without isomaltase activity (Fig. 4). (c) There is no mutual inhibition between sucrose and isomaltose, whereas such an inhibition is clearly present between sucrose and maltose; isomaltose and maltose; isomaltose and dextran; and palatinose and dextran (Table II). (d) At pH 9.6 and 37° sucrase and isomaltase are inactivated at different rates (Fig. 5).

The simplest explanation for these observations is that sucrase–isomaltase carries 2 substrate-specific groups. One group splits sucrose and maltose and the other isomaltose, palatinose, maltose, and also, but much more slowly, dextran. As Fig. 5 clearly shows, the inactivation of maltase at pH 9.6 does not follow first-order kinetics. It consists of at least 2 components, of which one is inactivated at the same rate as sucrase, and the other as isomaltase. If sucrase and isomaltase together account for the whole of the maltase activity in sucrase–isomaltase, and under the conditions of assay of maltase activity, sucrase would account for approx. $^2/_3$ and isomaltase for $^1/_3$ of the maltase activity.

Isolated sucrase–isomaltase has, therefore, at least 2 distinct 'active sites' which have different sensitivities towards inactivating agents. This situation is not the same as that found for muscle aldolase[31] (EC 4.1.2.7), carboxypeptidase[32] (EC 3.4.2.1) and pepsin[33] (EC 3.4.4.1) which carry one active site only, the specificity of which can be altered by discrete changes.

Obviously, both the mol. wt. and the number of active sites carried by an isolated component of a membrane (such as sucrase–isomaltase[24]) may depend on the method used to degrade the membrane. It seems meaningless to try to choose between 'two enzymes bound together' and 'an enzyme having two active sites'. For such catalytically active structures which are bound to, or are building blocks of a membrane, the term 'hyphezyme' (from ὑφή fabric) has been suggested[34]. Sucrase–isomaltase, as isolated here, would thus contain at least 2 hyphezymes.

Non-identity of intestinal sucrase and isomaltase has been previously reported in man[35,36], rat and hog[6]. The present results, while extending these observations, further suggest that sucrase and isomaltase are closely linked spatially. Since these

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two hyphezymes are under the same genetic control, at least in man[37], they apparently form both an anatomical–chemical and a genetic unit.

Isolated rabbit sucrase–isomaltase does not split L-leucyl-β-naphthylamide[38], α-L-glutamyl-β-naphthylamide, *p*-nitro-phenylphosphate[39], 6-bromo-2-naphthyl-α-glucoside*,[40], L-leucyl-glycine (0.01 M; Mn^{2+} or Mg^{2+} 0.001 M, pH 8.5), glycyl-

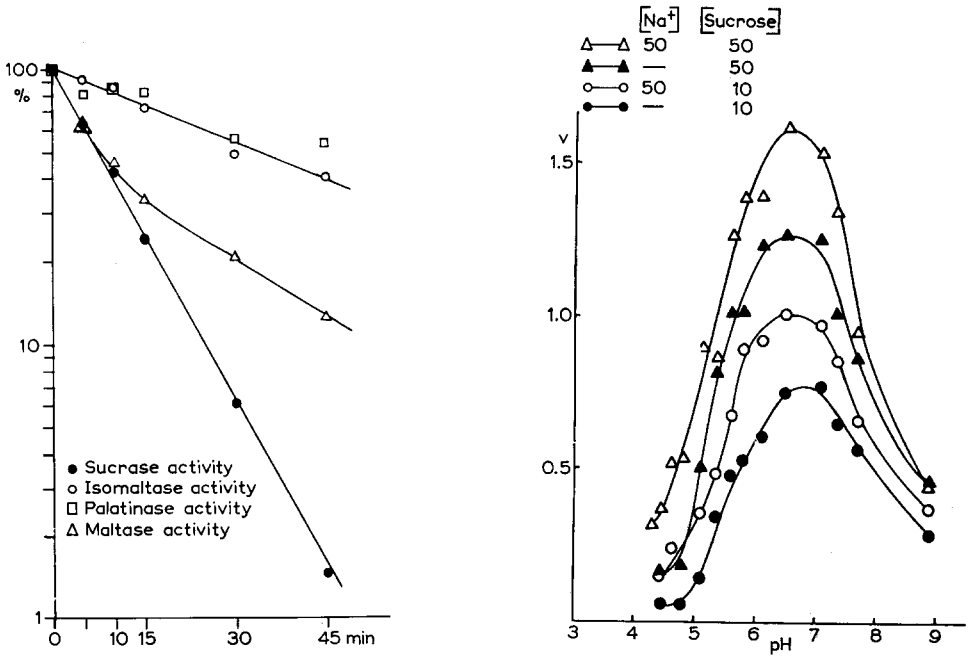

Fig. 5. Inactivation of sucrase–isomaltase at 37° and pH 9.6 (approx. 0.02 sucrase unit /ml in 0.05 M glycine–NaOH buffer). Samples were withdrawn and mixed with ice-cold 0.3 M sodium maleate buffer to yield a final pH of about 7. The enzyme activities were determined at the following substrate concentrations: sucrase, 0.05 M; isomaltase, 0.075 M; palatinase, 0.05 M; and maltase, 0.0083 M.

Fig. 6. pH–activity curves for sucrase. Buffers: pH 4.5–4.8, 0.05 M lithium acetate; pH 4.9–6.5, 0.05 M lithium maleate; pH 7–9, 0.05 M lithium phosphate. The concentrations of Na^+ (as NaCl) and of sucrose are in mM.

glycine (0.01 M; Co^{2+} 0.001 M, pH 7), triglycine (0.01 M, pH 7.5) or glycine-L-leucine (0.01 M, Mn^{2+} 0.001 M, pH 8.5). Neither does it have detectable mutarotase activity[42].

pH–activity curves, Na^+ activation, apparent K_m values

Fig. 6 shows the pH–activity curves in the presence and absence of Na^+, and at high and low substrate (sucrose) concentrations. The pH optimum, 6.8, is in agreement with that reported by CARNIE AND PORTEOUS[3] and by GITZELMANN, DAVIDSON AND OSINCHAK[8], and does not change either with Na^+ or substrate con-

* A hydrolytic activity as low as 1% of the sucrase activity could have been detected. This is at variance with that reported for rat sucrase[41], which also splits this substrate.

centration. The pH–activity curves of hamster sucrase, however, do vary with the Na^+ concentration[43].

Na^+ increases the apparent maximum velocity by some 20–30%, with little or no effect on the apparent K_m for sucrose. (In a number of experiments the ratio between the apparent K_m values with and without Na^+ ranged between 0.8 and 1.2.) This applies to both particle-bound (Fig. 7) and isolated rabbit sucrase, as well as to the human enzyme[44]. Sodium also activates intestinal sugar transport in the rabbit by increasing the maximal velocity with little or no effect on the apparent K_m (ref. 43). This observation is at variance with those on rat and hamster intestinal sucrases and on sugar transport systems, in which Na^+ activation is achieved by means of its large effect on apparent K_m values, with little or no change in the apparent maximum velocity[43,59].

Fig. 8 shows the determination of the sodium dissociation constant at pH 6.1 and at 2 different Li^+ concentrations. The same apparent K_{Na} is obtained at both Li^+ concentrations, indicating a lithium dissociation constant much larger than the Li^+ concentrations used. The presence of this cation can, therefore, be disregarded

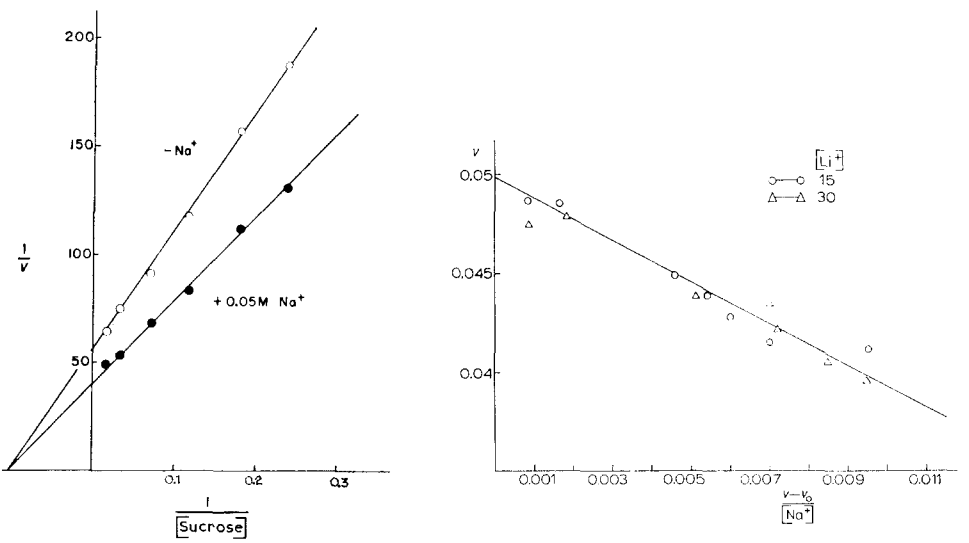

Fig. 7. Lineweaver–Burk plots of particle-bound sucrase activity with and without NaCl, in 0.0166 M Li^+ (maleate buffer), pH 6.6. Enzyme velocity (v) in μmoles hydrolysed substrate per min; substrate concentration in mM.

Fig. 8. Modified Eadie plot for the determination of the apparent sodium dissociation constant at two Li^+ concentrations (mM) in maleate buffer (pH 6.1); sucrose, 50 mM. v_0, enzyme velocity in the absence of Na^+. Na^+ and Li^+ concentrations expressed as mM.

at first approximation. The K_{Na} calculated from Fig. 8 is about 0.8 mM. It agrees well with the value of 0.75 mM, determined for particle-bound rabbit sucrase under similar conditions[43]. Apparently, therefore, solubilization and isolation have not brought about detectable changes in the activation by Na^+.

Fig. 9 illustrates the direct determination of the apparent K_K/K_{Na} ratio for isolated sucrase. The equation used has been derived elsewhere (number 1.90.1,

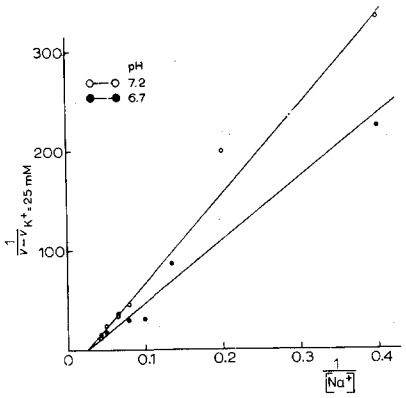

Fig. 9. Determination of the ratio between K^+ dissociation constant and Na^+ dissociation constant at two pH values (pH 6.7 in 0.0125 M sodium–potassium maleate buffer; pH 7.2 in 0.016 M sodium–potassium phosphate buffer). The sum $[Na^+] + [K^+]$ was kept constant (25 mM). Sucrose, 50 mM. $v_{K^+ = 25\,mM}$, velocity at $[K^+] = 25$ mM and $[Na^+] = 0$.

Table I in ref. 1). The sum of Na^+ and K^+ was kept constant (25 mM) and the reciprocal of the velocity, corrected for the velocity at $[Na^+] = 0$ and $[K^+] = 25$ mM, *i.e.*, $1/(v - v_{K^+ = 25\,mM})$ is plotted against $1/[Na^+]$. From the intercept on the $1/[Na^+]$ axis the K_K/K_{Na} ratio at both pH's considered (6.7 and 7.2) can be estimated to be 0.45. This ratio agrees closely with the corresponding ratio (0.6) which we have calculated in a similar way[1] from SCHULTZ AND ZALUSKY's data[45] on glucose-dependent and glucose-independent Na^+ transfer across rabbit intestinal mucosa.

We have suggested elsewhere[34] that the Na^+ site of sucrase is similar to, or identical with, the Na^+ site of the intestinal sugar carrier. Hence, Na^+ activation of isolated sucrase can be studied as a model of sodium activation of the sugar carrier. Investigations along these lines are in progress.

Valinomycin (0.04 μM) did not affect either the Na^+ activation or the K_m of sucrase.

At pH 6.8 the apparent K_m values for sucrose (Fig. 7, see also ref. 3) and for isomaltose are 8 mM and 5 mM, respectively.

Effect of Tris

Tris is a well-known inhibitor of intestinal disaccharidases[14,46–48]. The inhibition has been reported to be fully competitive for hog isomaltase ($K_i = 0.14$ mM, at pH 7.4, ref. 46), and for calf lactase ($K_i = 1.55$ mM, at pH 7.4, ref. 48). Since these reports have appeared, it has become known that sucrase and isomaltase are activated by Na^+ and inhibited by ammonium[34,43,44], and that polyols competitively inhibit glycosidases[49]. In principle, therefore, one may expect Tris, which is both a polyol and an amine, to inhibit sucrase by competing with Na^+ for the allosteric site, and/or with sucrose for the substrate site. Distinction between these two possibilities is important because of the relationship of Na^+ activation of sucrase with Na^+ activation of sugar transport[34] (see above).

For this purpose, neither hamster nor rat sucrase would be favourable enzymes, since Na^+ activation mainly affects the apparent K_m for sucrose[43]. In such a setting, a Na^+ competition affecting the apparent K_m for sucrose could be distinguished from

a fully competitive substrate inhibitor only with some difficulty. However, since rabbit and human sucrases are Na^+ activated through an increase in the maximum velocity, rather than a change in the apparent K_m, it is easier to distinguish kinetically between an allosteric effector, competing for the Na^+ site, and a fully competitive inhibitor, competing for the substrate site.

Fig. 10 shows that Tris is a fully competitive inhibitor of sucrase; the K_i for a fully competitive inhibition calculated at different concentrations of Tris is 0.4 mM at pH 6.7*. The effect of Tris is opposite to that of phlorizin. Phlorizin, a fully competitive inhibitor of intestinal sugar transport[50], does not affect intestinal sucrase[43]; Tris, a fully competitive inhibitor of sucrase (Fig. 10) does not affect sugar transport[51].

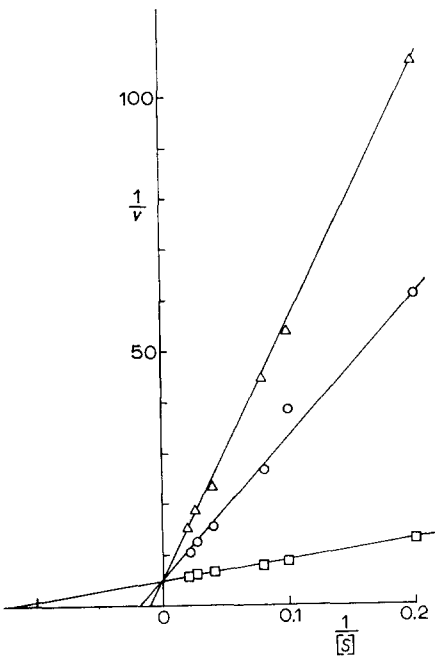

Fig. 10. Tris inhibition of sucrase in 0.03 M sodium maleate buffer (pH 6.7). □, no Tris; ○, 2 mM Tris; △, 5 mM Tris. For other details, see Fig. 7.

Furthermore, genetic disturbances in man are known which independently affect intestinal sucrase[52,53] or glucose absorption[54,55]. Therefore, while the Na^+ site of sucrase and the Na^+ site of the sugar carrier are similar if not identical (see above), the substrate sites of sucrase and sugar carrier are certainly not the same.

Lactate (0.02 and 0.05 M) had no effect on sucrase.

Effect of heavy metals

p-Cloromercuribenzoate (0.05 M) does not inhibit sucrase significantly. (It inhibits *Escherichia coli* β-galactosidase at concentrations as low as 10^{-8} M, ref. 56.)

* Another mechanism which could account for the inhibition by Tris could be that Tris, a polyol, may act as a very good acceptor for the transglucosidase activity. Besides the type of kinetics observed this possibility is rendered unlikely by the lack of extra spots in chromatograms obtained from the incubation mixtures (chromatography on Whatman No. 1, in *n*-butanol–5% aqueous ammonia; spots revealed with the periodate–benzidine reaction[60]).

TABLE III

EFFECT OF SOME HEAVY METALS ON SUCRASE ACTIVITY

Each incubation mixture contained 0.03 M sodium maleate buffer (pH 6.7), 0.05 M sucrose, 0.14 unit of sucrase per ml, and the heavy metal ions at the concentrations indicated below.

Metal ion	*Concn. (mM)*	*Sucrase activity as percentage of the control*
None	—	100
Hg^{2+}	0.1	0
Ag^{+}	0.1	0
Zn^{2+}	1	11.2
Co^{2+}	1	100
Ba^{2+}	1	100

Evidently, as with intestinal lactase[48], no free sulfhydryl group is necessary for enzymic activity.

Table III and Fig. 11 show the effect, or the lack of effect, of some metals on sucrase. The results indicate that group(s) having properties similar to those of imidazole might be necessary for sucrase to be catalytically active. This finding would agree with the shape and position of the pH–activity curves (Fig. 6) and with the results of LARNER AND GILLESPIE on photo-oxidation, heavy metal inhibition, and pH-dependence of hog intestinal isomaltase[46].

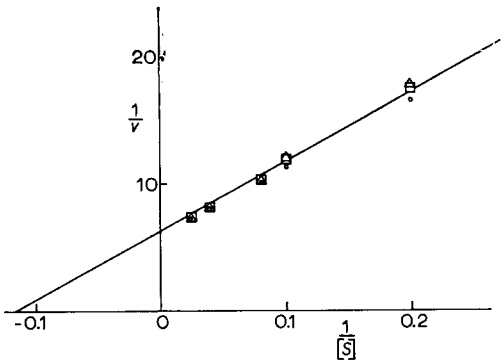

Fig. 11. Lack of inhibition of sucrase by Co^{2+} (1 mM (△)) or by Ba^{2+} (1 mM (□)) in 0.03 M sodium maleate buffer (pH 6.7). ○, no divalent metal present. For other details, see Fig. 7.

A double-displacement mechanism, of the type suggested for a number of other carbohydrases[57,58], could include the participation of an imidazole group and explain the liberation of α-glucose by sucrase and by isomaltase (*i.e.*, without inversion of the C_1 configuration of the glycone moiety)[42].

Nevertheless, the data available do not permit speculation as to whether imidazole group(s) actually take(s) part in the catalysis or is (are) simply necessary to stabilize the enzyme in a catalytically active form.

NOTE ADDED IN PROOF (Received July 31st, 1967)

More recent data, obtained under somewhat different conditions, show that pullulanase is, in fact, retained by Sephadex (K. WALLENFELS *et al.*, personal communication, 1967).

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