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A HYDROPHOBIC FORM OF THE SMALL-INTESTINAL SUCRASE-ISOMALTASE COMPLEX

HANS SIGRIST , PETER RONNER and GIORGIO SEMENZA

Laboratorium für Biochemie II, Eidgenössische Technische Hochschule, Universitätstrasse 16, CH 8006 Zürich (Switzerland)

Summary

A large scale preparation of brush border membranes is described. Solubilized by either papain or Triton X-100, the sucrase-isomaltase complex is purified in a three-step procedure, including differential centrifugation, Sephadex G-200 and DEAE-cellulose chromatography. Detergent solubilized and protease solubilized sucrase-isomaltase differ in the tendency to aggregate but not in enzymatic characteristics. The chemical composition and the molecular weight of the two enzyme complexes are almost identical. Limited digestion of the Triton-solubilized sucrase-isomaltase complex by papain produces a protein electrophoretically indistinguishable from papain-solubilized sucrase-isomaltase together with low molecular proteolytic fragments.

Introduction

Due to the interest in the role of small intestinal sucrase-isomaltase as a translocator for sugars both in the original brush border membrane [1—3] and in artificial membranes [4, 5], this di-enzyme complex has been the object of a number of studies in the present laboratory and in others. Sucrase-isomaltase can be solubilized either by Triton X-100 or by papain [6—10] and obtained in a homogeneous form by ammonium sulfate fractionation and affinity chromatography on Sephadex G-200 [8, 9]. This paper reports: (i) a large scale preparation of brush border membranes, (ii) isolation of the sucrase-isomaltase complex after either papain or Triton solubilization, (iii) a first comparison between papain-solubilized and Triton-solubilized sucrase-isomaltase.

Material and Methods

All reagents were of the highest commercial grade available and, except

as indicated below, were used as obtained. Triton X-100 was obtained from Sigma Chemical Co. Bovine serum albumin (M_r = 68 000), ovalbumin (M_r = 45 000), human γ-globulin (H-chain, M_r = 50 000) (Schwarz/Mann, New York) and papain-solubilized sucrase-isomaltase (140 000 apparent subunit molecular weight [8, 29]) were used as marker proteins in sodium dodecyl sulfate polyacrylamide gel electrophoresis. Autolysis products in the papain crystal suspension (Boehringer, Mannheim) were separated by Sephadex G-25 chromatography. Two preparations of immobilized papain were used: Enzyte Agarose Papain suspension (Miles Laboratories, England) and Enzygel-Papain lyophilisate (Boehringer, Mannheim). Both preparations were thoroughly washed by repeated centrifugation. Iodoacetic acid (Merck) was recrystallized from hot benzene.

Sucrase (sucrose α-glucohydrolase, EC 3.2.1.48) and isomaltase (EC 3.2.1.10) were assayed as described previously with 33 mM sucrose or 33 mM palatinose as substrates [6]. The liberated glucose was determined by the Tris-glucose-oxidase method [11], 50% sulfuric acid being used for color stabilization [12].

In the absence of Triton, protein was determined according to Lowry et al. [13] or by measuring the absorbance at 280 nm. The protein content of Triton-containing solutions was determined by the modified Lowry method as described by Ji [14]. Triton X-100 was assayed by the procedure of Garewal [15]; The lipid content was determined with the phosphoric acid/vanilla reagent according to Zöllner and Kirsch [16], total phosphorus by the method of Chen et al. [17].

Amino acid analysis: 0.5 mg samples of the protein were hydrolyzed in duplicates in 1 ml 6 M HCl containing 0.01% phenol under vacuum at 100°C for 24, 48, 72 h respectively. Norleucine was used as internal standard. The amino acids were determined on a BC 200 analyzer (Calbiochem). Total cysteine plus half-cystine was determined after denaturation (6 M guanidine-HCl), reduction with dithioerythritol (50 mM) and carboxymethylation of the protein with iodoacetic acid (150 mM), as carboxymethylcysteine. The protein samples were hydrolyzed in 6 M HCl for 24 h at 100°C. Tryptophan was determined by the method of Edelhoch [18]. Sodium dodecyl sulfate gel electrophoresis was performed in 0.1% sodium dodecyl sulfate, 0.1 M sodium phosphate buffer, pH 7.2, in 5.5% polyacrylamide gels [19]. Before electrophoresis the desalted protein samples were incubated at 37°C in 1% sodium dodecyl sulfate for 3 h and dialyzed overnight against diluted electrode buffer (0.01% sodium dodecylsulfate, 0.01 M sodium phosphate buffer, pH 7.2). After electrophoresis, fixation, amidoblack staining and destaining the gels were scanned at 580 nm with a ISCO gel scanner.

Polyacrylamide gel electrophoresis in the absence of sodium dodecyl sulfate was performed according to the procedure of Ornstein and Davis [20, 21] as described by Maurer [22] (Tris/glycine system, pH 8.3, 7% acrylamide). Both spacer and sample gels were omitted and the sample (20—100 μl) was mixed with one drop of glycerol and 3 μl of bromophenol blue [19].

Experimental procedure

Preparation of brush border membranes

Rabbit small intestine, obtained immediately after sacrifice, rinsed with physiological saline, inverted and washed with ice-cold physiological saline, was stored at –20°C. In a standard procedure 150 g frozen intestines were cut in 1 cm tubes and thawed in 500 ml Tris/D-mannitol buffer, (300 mM D-mannitol, 12 mM Tris/HCl, pH 7.1). All the following operations were performed at 4°C. In a beaker, the suspended intestines were treated with a vibrator, (Vibro Mischer E 1, Chemap, Switzerland) fitted with a 6.5 cm diameter perforated endplate, for 90 s at full speed. The intestines were then retrieved by filtration through a porcelain Büchner funnel. The filtrate was diluted with cold water to a final concentration of 50 mM D-mannitol, 2 mM Tris/HCl; 800-ml aliquots of filtrate were homogenized for 3 min at full speed in a Waring blendor. Crystalline $CaCl_2$ was added to a final concentration of 10 mM. The suspension was allowed to stand 10 min and thereafter centrifuged for 7 min at 2000 x *g*. The pellet (P_1) was discarded. The supernatant (S_1) was then recentrifuged at 35000 x *g* for 30 min. The supernatant (S_2) was discarded, the pellet (P_2), containing membrane fragments (approx. 450 mg) was resuspended in water or stored at –20°C.

The flow scheme of the preparation of rabbit brush border membranes is given in Fig. 1.

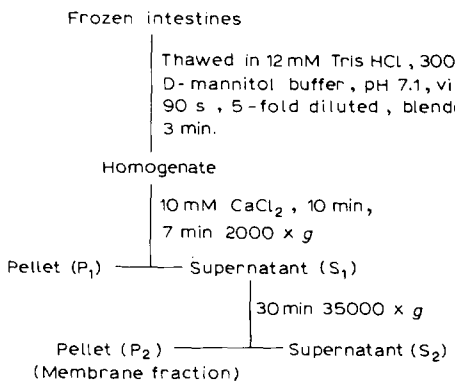

Fig. 1. Flow sheet of the preparation of brush border membranes from rabbit small intestine.

Isolation of papain-solubilized sucrase-isomaltase

Papain solubilization. Activation of papain: Commercial papain (crystal suspension in freshly prepared 50 mM potassium phosphate buffer, pH 6.8, containing 5 mM cystein hydrochloride, 1 mM EDTA was saturated with nitrogen for 30 min at room temperature.

The membrane fraction was suspended in water or 10 mM potassium phosphate buffer pH 6.8 (30 mg protein/ml) and homogenized by 3—4 strokes of a motor-driven Teflon-glass homogenizer. The homogenate, diluted with cysteine/EDTA buffer (final concentration 5 mM cysteine hydrochloride, 1 mM

EDTA, 50 mM potassium phosphate buffer, pH 6.8; protein 12 mg/ml), was incubated under nitrogen at 37°C for 30 min with activated papain. 0.09 unit papain was used per mg brush border membrane protein. Papain was inactivated by addition of sodium iodoacetate, pH 6.8, to a final concentration of 10 mM. The unsolubilized membranes were immediately separated by centrifugation at 90 000 x *g* for 60 min. The pellet (P_3P) was discarded, the supernatant (S_3P) concentrated during 16 h at 150 000 x *g*. 90—95% of the concentrate supernatant was carefully pipetted off, the viscous concentrate was resuspended in the residual solution and applied to a Sephadex G-200 column.

Purification of the papain-solubilized sucrase-isomaltase. 20 ml of the concentrate, obtained as described above from 600 g frozen intestine were applied on a Sephadex G-200 column (5 cm diameter x 64 cm) and eluted with 10 mM potassium phosphate buffer, pH 6.8. The sucrase active fractions, which emerged after the total volume of the column, were concentrated on a DEAE-cellulose column (2 cm x 10 cm) as described [8] and eluted with 300 mM potassium phosphate buffer, pH 6.8. Further concentration was achieved by high speed (150 000 x *g*) centrifugation overnight.

Isolation of Triton X-100 solubilized sucrase isomaltase

Triton X-100 solubilization. 1.8 g brush border membrane (starting material: 600 g frozen intestine) was suspended in 225 ml water and diluted with the same volume of cold Triton/phosphate buffer to a final concentration of 0.5% Triton X-100, 0.1 M NaCl, 10 mM sodium phosphate pH 7.0. The final protein concentration was approximately 4 mg/ml. The membranes were kept at 4°C for 90 min with occasional stirring. Unsolubilized particles (pellet P_3T) were separated by centrifugation, 60 min at 90 000 x *g*. The supernatant (S_3T) was concentrated during 16 h by centrifugation under the same conditions. Approximately 9/10 of the supernatant was pipetted off and discarded. The viscous yellow sediment was resuspended in the residual supernatant and immediately chromatographed on Sephadex G-200.

Isolation. The concentrate (40—50 ml), as obtained above, was applied to a Sephadex G-200 column (6 cm diameter x 35 cm), preequilibrated with 0.5% Triton X-100, 0.1 M NaCl, 10 mM sodium phosphate buffer, pH 7.0. The column was developed with the equilibration buffer at 4°C. A flow rate of 15—20 ml/h was maintained by a peristaltic pump or by hydrostatic pressure. The sucrase-isomaltase complex was eluted starting from 1.5-fold up to a 4-fold column volume (V_t). The eluate was concentrated immediately on a DEAE-cellulose column (1.8 cm diameter x 8 cm), equilibrated with the same elution buffer as used in the Sephadex column, at a flow rate of 30 ml/h. The cellulose column was washed for 2 h with the same buffer. Protein eluted sharply with 0.3 M potassium phosphate buffer containing 0.5% Triton X-100, 0.1 M NaCl at the same flow rate. The sucrase active fractions were concentrated by centrifugation for 16 h at 100 000 x *g*.

Removal of Triton X-100. Triton X-100 was removed by acetone precipitation [9] as follows. The protein solution containing 1 mg protein/ml Triton/phosphate buffer was mixed with twice its volume ice-cold acetone. The precipitate formed was spun down at 1250 x *g* for 3 min. The sediment

was dissolved in water and reprecipitated twice as above. The final sediment was dissolved in water up to a protein concentration of 10 mg/ml. In a standard procedure, 1 ml of the concentrated protein solution was applied to a Sephadex G-25 column (1.8 cm diameter x 24 cm) equilibrated with the desired buffer system. The column was eluted with a flow rate of 2.5 ml/10 min. Fig. 2 summarizes schematically both solubilization and purification procedures.

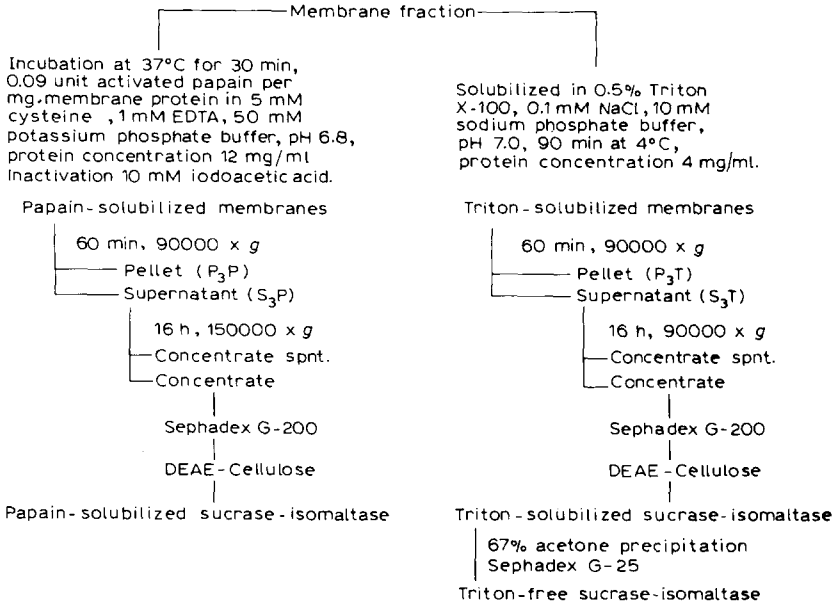

Fig. 2. Purification of the sucrase-isomaltase complex, solubilized by either papain or Triton X-100.

Papain treatment of Triton-solubilized sucrase-isomaltase

Triton-solubilized sucrase and papain (crystal suspension) were purified before use on Sephadex G-25 (washed with 1 M NaCl, 20 mM sodium phosphate buffer pH 7.0 and preequilibrated with water overnight). 5.5 mg papain in 32.5 ml water was combined with 11 ml of a freshly prepared solution of 5 mM cysteine hydrochloride, 1 mM EDTA pH 7.0 (adjusted with NaOH). The papain-containing solution was then stored 20 min at room temperature and 10 min at 4°C. The limited digestion was started by addition of 22 mg (11.5 ml) Triton-solubilized sucrase. The final concentrations were: Triton-solubilized sucrase 0.4 mg/ml, papain 0.1 mg/ml, cysteine hydrochloride 1 mM, EDTA 0.2 mM. The digestion performed during 16 h at 4°C was stopped by the addition of iodoacetic acid (final concentration 2 mM, adjusted to pH 7.0 with NaOH just before use). The incubation medium was immediately frozen in liquid nitrogen and lyophilized overnight.

Separation of the digestion products

The lyophilized digestion products were applied to a Sephadex G-25 column (1.6 cm diameter x 27 cm) equilibrated with 0.1% $(NH_4)_2CO_2$. The

low molecular weight components were pooled and rechromatographed on Sephadex G-10 (0.8 cm diameter x 9 cm) in 0.625 M formic acid. Further separation was achieved by descending paper chromatography on Whatman 1, in the organic phase of a 1-butanol/acidic acid/water (4 : 1 : 5, by vol.) mixture.

Results and Discussion

The present paper reports (a) a procedure for the rapid large-scale preparation of brush border membranes from rabbit small intestine, based on a previously reported method [23]; (b) a detailed prescription for the solubilization and isolation of the small intestinal sucrase-isomaltase by the use of Triton X-100; (c) a further simplification and improvement of the procedures for papain-solubilization and isolation of the small intestinal sucrase-isomaltase complex, and (d) data comparing properties of the sucrase-isomaltase complexes solubilized by either papain or Triton X-100. Each of the three preparative procedures described here have now been in routine use in our laboratory and have proved to be remarkably reproducible. Their merits, as compared to previous procedures can be summarized as follows.

Preparation of brush border membranes

The procedure for the preparation of brush border membranes avoided scraping of the intestinal mucosa and allowed frozen material to be utilized, with obvious practical advantages. Routinely, about 2 g of isolated brush border membranes could be prepared from 600 g tissue in 8 h. These brush border membranes were a very suitable starting material for the solubilization and isolation of their components. The specific activity of the sucrase-isomaltase, in particular, was already as high as 1/10 of the homogeneous [8] sucrase-isomaltase complex (Table I). The enzymatic characteristics of this fractionation procedure together with the transport properties of the vesiculated membranes will be published elsewhere [24].

TABLE I

SUMMARY OF A REPRESENTATIVE PREPARATION OF BRUSH BORDER MEMBRANES FROM FROZEN RABBIT SMALL INTESTINE

The specific activity is expressed as international units (I.U.) per mg protein. 1 I.U. of sucrase liberates 1 μmol glucose per min. from sucrose under the conditions described in Materials and Methods.

Fraction	Sucrase		Purification
	Recovery (%)	Spec. activity (units/mg)	
Homogenate	100	0.1	1
Supernatant (S_1)	76	0.15	1.5
Pellet (P_1)	24	0.047	
Supernatant (S_2)	7	0.02	
Pellet (P_2)	69	1.32	13.2

Triton X-100 solubilization

Solubilization of the membrane fraction was performed essentially as described by Takesue et al. [9]. The membranes (final concentration 4 mg/ml) were solubilized at 4°C for 90 min. Under the conditions chosen, 27% of the

TABLE II

TRITON X-100 SOLUBILIZATION AND PURIFICATION OF THE SUCRASE-ISOMALTASE COMPLEX

The protein and sucrase activity values listed are the average of determinations from 5 experiments. Palatinase activities from one representative preparation are given

Fraction	Protein (%)	Sucrase activity		Palatinase activity	
		Spec. act. (units/mg)	Recovery (%)	Spec. act. (units/mg)	Recovery (%)
Membrane fraction	100	1.35	100	0.16	100
Membrane fraction after Triton X-100 treatment	93.4	1.37	92.8	0.16	105
Supernatant 90 000 x *g*, 60 min	63.5	1.19	54.3	0.23	69.1
Pellet 90 000 x *g*, 60 min	29.6	1.24	27.2	0.18	38.6
After concentration at 90 000 x *g* for 16 h					
Supernatant	28.7	0.16	3.3	0.01	1.7
Concentrate	53.9	1.96	50.9	0.26	51.0
After Sephadex G-200 and DEAE-cellulose	2.2	9.3	16.6	1.1	13.2
After removal of Triton X-100 (Triton-free sucrase)	1.9	12.6	17.5	1.5	13.8

sucrase activity remained insoluble (Table II). This fraction (P_3T) was discarded since the specific activity was lower than in the untreated membranes. By subsequent centrifugation for 16 h the sucrase-isomaltase complex was not only concentrated but also separated from small molecular weight proteins. This step replaced the ammonium sulphate precipitation in Takesue's procedure. Its application was limited by the volume capacity of the high speed rotor.

Papain solubilization

The papain solubilization of the membrane fraction may be controlled by varying the incubation time or by the amount of papain used per mg membrane protein. With respect to a large scale preparation of sucrase-isomaltase, the final conditions were selected towards both optimal incubation time and the amount of papain needed. Using activated papain instead of an untreated papain suspension in cysteine/EDTA buffer [8], the necessary amount of papain producing the same degree of solubilization (measured by the liberation of sucrase) was lowered by a factor of 50. The susceptibility of papain to oxygen in presence of low concentration of cysteine was described by Sluyterman [25]. Papain is inactivated irreversibly by iodoacetate at slightly alkaline or neutral pH [26, 27].

Sephadex G-200 and DEAE-cellulose chromatography

The purification of the papain-solubilized sucrase-isomaltase complex by affinity chromatography on Sephadex G-200 and the concentration on DEAE-cellulose were performed as described previously [8]. The enzyme complex showed the same properties. Identical elution patterns were obtained. The purification procedure is summarized in Table III.

The affinity of the sucrase-isomaltase complex for Sephadex G-200 [6] was retained in presence of the detergent (Fig. 3). However, the amount of sucrase not retained and eluted with the void volume of the column, was related to the degree of purification of the applied sample. A low specific sucrase activity of the applied material ($\leqslant$ 1.5 units/mg protein) resulted in a small yield of retarded enzyme complex. The ionic properties of DEAE-cellulose in presence of Triton X-100 were maintained.

TABLE III

PAPAIN-SOLUBILIZATION AND PURIFICATION OF THE SUCRASE-ISOMALTASE COMPLEX

The listed data are derived from a preparation starting with 2.3 g brush border membrane protein

Fraction	Protein (%)	Sucrase activity		Palatinase activity	
		Spec. act. (units/mg)	Recovery (%)	Spec. act. (units/mg)	Recovery (%)
Membrane fraction	100	0.78	100	0.09	100
Membrane fraction after papain treatment	74.0	1.22	115.8	0.15	121
Supernatant 90 000 x *g*, 60 min	26.4	2.74	92.2	0.36	98
Pellet 90000 x *g*, 60 min	40.3	0.37	19.4	0.05	21.4
After concentration at 150 000 x *g* for 16 h					
Supernatant	6.9	0.0		0.0	
Concentrate	13.5	4.63	79.9	0.57	82.5
After Sephadex G-200	6.5 [a]		70.5 [a]		70.4 [a]
After DEAE-cellulose	3.8	11.41	55.1	1.47	59.5

[a] Values calculated from the elution diagram.

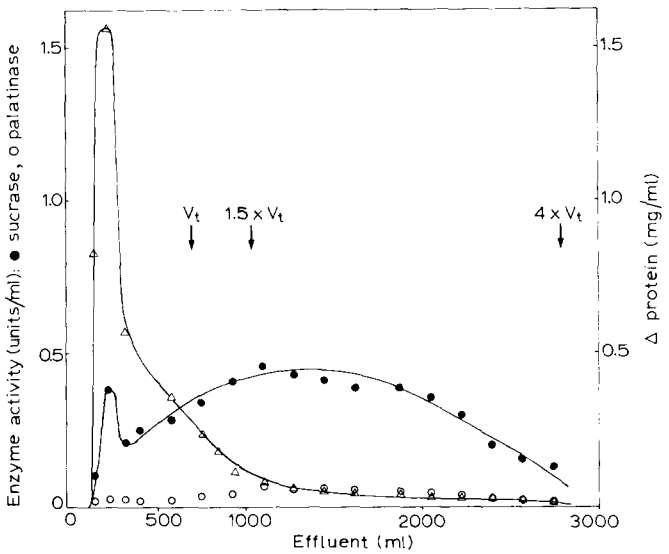

Fig. 3. Sephadex G-200 chromatography of Triton X-100 solubilized sucrase-isomaltase at 4°C. ●, sucrase activity; ○, palatinase activity; △, protein [14]. For details, see Experimental Procedure.

Removal of Triton X-100

By Garewal's method [15] no Triton X-100 could be detected in sucrase-isomaltase after three acetone precipitations. In addition, this "Triton-free" sucrase-isomaltase did not increase the permeability of mannitol entrapped in single-shell (egg-lecithin/phosphatidic acid) liposomes. (Vögeli, H., Brunner, J. and Semenza, G., manuscript in preparation). Thus if Triton X-100 is at all present in the final preparation of Triton-solubilized sucrase-isomaltase, its amount must be very small. As judged by acrylamide gel electrophoresis, acetone treatment did not change the aggregation properties of papain-solubilized sucrase-isomaltase.

Sephadex G-25 chromatography was carried out after the acetone precipitation in order to remove both remaining acetone and phosphate and to change buffer systems.

Criteria of purity of papain-solubilized and Triton-solubilized sucrase-isomaltase

Papain-solubilized sucrase-isomaltase as obtained by the procedure reported here, yielded a single band in acrylamide gel electrophoresis, having the same mobility as the sucrase-isomaltase complex,isolated according to Cogoli et al. [8]. Triton-solubilized sucrase-isomaltase in the absence of sodium dodecylsulfate did not penetrate the polyacrylamide gel. (Tris/glycine system pH 8.3, 7% acrylamide; phosphate system pH 7.2, 5.5% acrylamide). However, in the sodium dodecylsulfate-containing phosphate system, Triton-solubilized sucrase-isomaltase migrated as a single band.

In the analytical ultracentrifuge, Triton-solubilized "Triton-free" sucrase-isomaltase yielded two Schlieren peaks. Their sedimentation coefficients (s_{20}) were 15.6 S (small peak) and 47.0 S for the large asymmetrical peak presum-

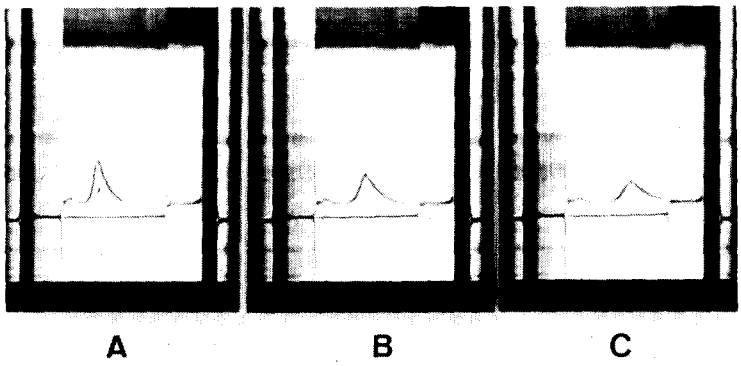

Fig. 4. Sedimentation pattern of the Triton-solubilized sucrase-isomaltase complex. 6.2 mg/ml sucrase-isomaltase in 10 mM potassium phosphate buffer, pH 6.8. The analysis was carried out on a Beckman E ultracentrifuge. Rotor speed 40 000 rev./min, temperature 20°C, angle of Schlieren diaphragm 60°. Pictures taken 4 min (A), 8 min (B) and 12 min (C) after reaching maximum speed.

ably corresponding to polydisperse aggregates (Fig. 4). Under comparable conditions papain-solubilized sucrase-isomaltase sedimented as a single Schlieren band with a sedimentation coefficient of 9.3 S [28]. The final preparations of both papain-solubilized and Triton-solubilized sucrase-isomaltase had the same specific activity as reported previously for the papain-solubilized enzyme [8].

Characteristics of Triton-solubilized sucrase-isomaltase

Sodium dodecyl sulfate gel electrophoresis. Sodium dodecyl sulfate-polyacrylamide electrophoresis yielded a single band with an apparent molecular weight of approximately 140 000 daltons for both Triton-solubilized and papain-solubilized sucrase-isomaltase (the latter confirmed our previous observations for the papain-solubilized enzyme prepared by a slightly different

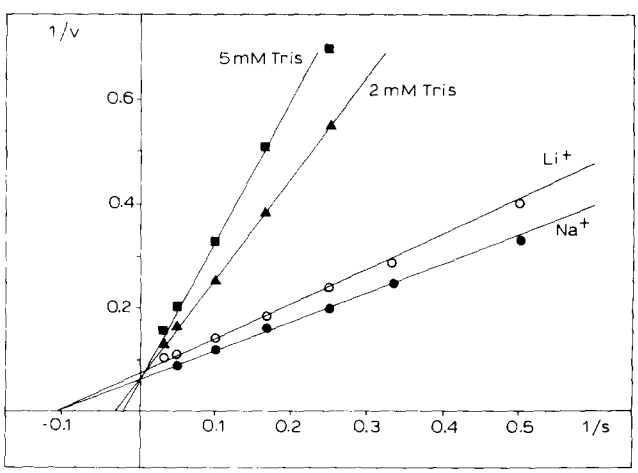

Fig. 5. Enzymatic characteristics of the Triton-solubilized sucrase-isomaltase complex. Lineweaver-Burk plot of the sucrase activity in presence of 33 mM Li^+ (○) (lithium maleate, pH 6.8) or 33 mM Na^+ (●) (sodium maleate, pH 6.8). Tris inhibition is shown using 2 mM (▲) and 5 mM (■) Tris/HCl in 33 mM sodium maleate buffer pH 6.8. The enzyme velocity (v) is expressed in μmol glucose liberated per min and mg protein. Substrate concentration (s) in mM.

procedure [29]). This indicated that papain-solubilization did not grossly alter the apparent molecular weight of the subunits.

Stability, Na^+-activation, apparent K_m and Tris-inhibition. In the presence of Triton X-100, sucrase-isomaltase could be stored in 0.02% sodium azide at 4°C or at –20°C for several weeks.

The Na^+-activation of the detergent-solubilized sucrase is shown in Fig. 5. As in the papain-solubilized complex [6], the sucrase activity of the Triton-solubilized complex was enhanced 20—30% by Na^+. The apparent K_m calculated from the Lineweaver-Burk plot (8.4 mM) was in excellent agreement with that of papain-solubilized sucrase (8.5 mM) (Fig. 5).

The Triton-solubilized sucrase-isomaltase complex was also fully competitively inhibited by Tris. An apparent K_i of 0.7 mM was determined at pH 6.8.

Chemical composition. The amino acid and amino sugar compositions of the detergent- and the protease-solubilized enzyme complex are listed in Table IV. The differences between the two forms of the enzyme complex are not significant, which is hardly surprising in view of the large molecular weight. No phosphate was detected ($\leqslant$ 0.5 mol phosphate/220 000 mol.wt). By the phosphoric acid/vanillin method a maximum "lipid content" of 2—4% was estimated. However, this must be regarded as an upper limit, since the carbohydrates originating from the glycoprotein (see Table IV) probably produced erroneously high results.

TABLE IV

AMINO ACID AND AMINO SUGAR COMPOSITION OF THE SUCRASE-ISOMALTASE COMPLEX

The values for amino acid content are, unless otherwise indicated, averages from 24, 48 and 72 h hydrolysates. The values for papain-solubilized sucrase-isomaltase are taken from Cogoli et al. [29].

Amino acid	Papain sucrase residues/100 000	Triton sucrase residues/100 000
Aspartic acid	121	122
Threonine	72 [a]	75 [a]
Serine	46 [a]	48 [a]
Glutamic acid	92	92
Proline	53	52
Glycine	54	55
Alanine	45	50
Valine	50	50
Methionine	20	20
Isoleucine	55	55
Leucine	67	68
Tyrosine	49	46
Phenylalanine	51	51
Lysine	27	27
Histidine	25	24
Arganine	46	48
Cysteine and half-cystine	13	12 [b]
Tryptophan	23	28 [c]
Glucosamine	31 [e]	31 [d]
Galactosamine	5 [e]	10 [d]

[a] Extrapolated to zero time of hydrolysis.
[b] Determined as carboxymethylcysteine.
[c] Determined according to Edelhoch [18].
[d] Extrapolated to zero time from 24 and 48 h 6 M HCl hydrolysates.
[e] Hydrolysis in 4 M HCl for 4 h at 110°C.

Limited digestion of Triton-solubilized sucrase by papain

The protease-solubilized and the detergent-solubilized small intestinal sucrase differed in their mobility in the Tris/glycine polyacrylamide gel system (Fig. 6). Sucrase activity was not affected by limited papain treatment. Therefore the digestion was monitored by: the disappearance of the aggregated enzyme and the simultaneous formation of "papain-sucrase" (as defined by its electrophoretic mobility) without loss of sucrase activity. A molar ratio 2:1 papain (M_r = 23 000)/Triton sucrase-isomaltase (M_r = 220 000) was found to be appropriate for the conditions chosen (1 mM cysteine hydrochloride, 0.2 mM EDTA, 16 h, 4°C, pH 7.0). The mobility of Triton-solubilized sucrase-isomaltase was not altered by any of the following conditions: (a) incubation without papain, (b) incubation in presence of 1 mM 2-mercaptoethanol or

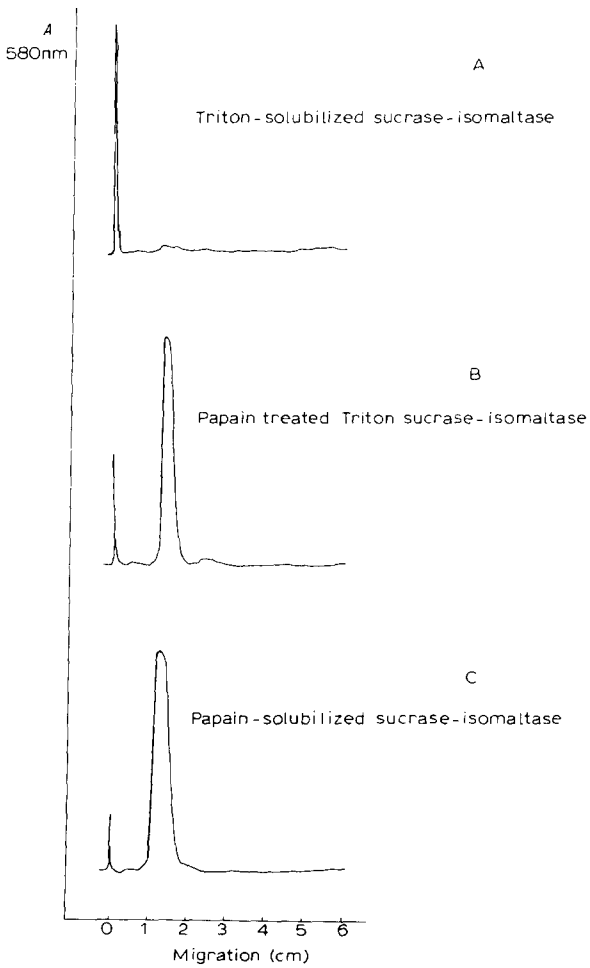

Fig. 6. Electrophoretic mobility in acrylamide gels. (A) Triton-solubilized sucrase-isomaltase (60 μg), (B) Papain treated Triton-solubilized sucrase-isomaltase (40 μg) and (C) Papain-solubilized sucrase-isomaltase (35 μg). The conditions for the limited digestion of Triton-solubilized sucrase-isomaltase are described under Experimental Procedure. 7% acrylamide gels were run for 40 min at 4 mA/gel in the Tris/glycine system pH 8.3. The isoelectrical point of papain is 8.75 [30]. Therefore it did not enter the gel under the chosen conditions.

1 mM dithioerythritol (final concentrations, without papain), (c) incubation with agarose-trapped, rather than soluble papain, (d) incubation at various ionic strengths between 0.002 and 0.72 in the absence of either papain or thiols.

In addition to the "papain-sucrase" (see above), limited papain digestion of Triton-solubilized sucrase-isomaltase produced some low molecular weight fragments, the isolation of which is described under Experimental Procedure. Their characterization is presently in progress. The overall amino acid composition of the fragments is, however, clearly more hydrophobic than that of either Triton-solubilized or papain-solubilized sucrase-isomaltase. The polarity (percentage of the polar amino acid residues as defined by Capaldi and Vanderkooi [31]) of these released peptides is approximately 29.9%; that of either Triton-solubilized or papain-solubilized sucrase-isomaltase is 47.2%. In agreement with these results Takesue [9] reported the existence of an additional antigen determinant in the Triton-solubilized sucrase-isomaltase.

Although further work is needed on the properties and size of hydrophobic areas in sucrase-isomaltase, it is already apparent that the Triton-solubilized enzyme is more hydrophobic than its papain-solubilized form. In fact, Triton-solubilized sucrase-isomaltase aggregates strongly in water solution (Figs 4 and 6) and hydrophobic peptides are released upon controlled papain digestion. Due to the known broad substrate specificity of this protease, we cannot state at present whether these hydrophobic fragments arise from one or more major hydrophobic peptides. The possibility that a hydrophobic area of the protein inserts into the membrane cannot be excluded.

Similar observations were reported by Nathenson and Cullen [32] for mouse H-2 alloantigens solubilized with the detergent NP 40 and papain. A transformation of the detergent extracted form of cytochrome b_5 (M_r = 16 000 [33]) and cytochrome b_5 reductase (M_r = 43 000 [34]) by limited protease treatment was demonstrated by Spatz and Strittmatter.

In both cases a hydrophobic segment was split off, whereas the enzymatic activities were maintained. The fragments contain 25—30% of the total amino acid residues. The localization of the major glycoprotein (M_r = about 50 000, 60% carbohydrate) in the human erythrocyte membrane is well documented [35]; 23 predominantly hydrophobic amino acids (11.3%) of the high polar membrane protein span the lipid bilayer [36]. In the case of the much larger sucrase-isomaltase (M_r = 220 000 [28, 29]) the loss of a hydrophobic peptide of the size reported for glycophorin or cytochrome b_5 could not be detected by the difference in amino acid composition of the Triton-solubilized and papain-solubilized enzymes.

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