Isolation of a Detergent-Solubilized Maltase/Glucoamylase from Rat Intestine and its Comparison with a Maltase/Glucoamylase solubilized by Papain

Lilian M. Y. LEE, Antonietta K. SALVATORE, Peter R. FLANAGAN and Gordon G. FORSTNER
*Research Institute, The Hospital for Sick Children, University of Toronto, Toronto, Ontario,
Canada M5G 1X8*

(Received 22 October 1979)

Maltase/glucoamylase from the rat intestinal brush-border membrane was solubilized by homogenization of the intestinal mucosa in buffer containing 0.5% Triton X-100. After removal of the detergent with butan-1-ol, the enzyme was purified by chromatography on Sepharose 4B and DEAE-cellulose. The final specific activity was 70.3 units/mg of protein in six preparations, comparing favourably with the specific activity of 65.0 units/mg of protein of a pure papain-solubilized maltase/glucoamylase previously isolated and characterized by us [Flanagan & Forstner (1978) *Biochem. J.* 173, 553–563]. The two enzymes were compared. Both migrated as single bands with the same mobility on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, were eluted at the same volume from Sepharose 4B, and had the same sedimentation pattern in mannitol gradients. The amino acid composition was similar; content of total apolar residues differed by 1.0 mol%. Antibodies prepared against either enzyme gave identical precipitin lines with each. Neither enzyme bound tritiated Triton X-100. The only difference noted was the tendency of the detergent-solubilized enzyme to aggregate on storage, whereas the papain-solubilized enzyme remained unchanged. Both enzymes had two *N*-termini, glycine and arginine. When the two enzymes were dissociated by boiling in sodium dodecyl sulphate, each exhibited the same five species on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. Single *N*-termini were found in the two smaller species, 1 (glycine) and 2 (arginine), whereas larger species (3–5) had both *N*-terminal amino acids. Both the Triton- and papain-solubilized enzymes appear to be oligomers of species 1 and 2, indicating that the native enzyme contains two subunit types. Aggregation in aqueous solutions does not depend on a proteolytically susceptible peptide fragment at the *N*-terminus of either subunit.

Maltase (α -D-glucoside glucohydrolase, EC 3.2.1.20/glucoamylase (1,4- α -D-glucan glucohydrolase, EC 3.2.1.3) is one of a group of oligo-disaccharidases which are constituents of the intestinal brush-border membrane (Miller & Crane, 1961; Dahlqvist & Thomson, 1963; Eichholz & Crane, 1965; Forstner *et al.*, 1968; Alpers & Solin, 1970). It is easily solubilized from the membrane in good yield by papain digestion (Auricchio *et al.*, 1963; Eichholz, 1968; Forstner, 1971). Purified preparations of the papain-solubilized enzyme have been studied by several investigators in a variety of species, including the monkey (Seetharam *et al.*, 1970), suckling rat (Schlegel-Haueter *et al.*, 1972),

adult rat (Kolinska & Kraml, 1972; Flanagan & Forstner, 1978, 1979), human (Kelly & Alpers, 1973) and rabbit (Sivakami & Radhakrishnan, 1973).

Several authors have shown that maltase/glucoamylase can be solubilized by detergents (Maroux *et al.*, 1973; Louvard *et al.*, 1975). In contrast with the work on the papain-solubilized enzyme, however, there is only one report of an attempt to purify a detergent-solubilized maltase-glucoamylase (Maroux & Louvard, 1976). These authors obtained a preparation which was purified 10-fold with respect to purified pig membrane vesicles, and the preparation gave a single band on gel electrophoresis. Hydrophobic peptides of approximate molecular weights 8000–10000 could be removed

Abbreviation used: SDS, sodium dodecyl sulphate.

by trypsin, suggesting that the enzyme contained a hydrophobic component which might bind the catalytic moiety to the lipid matrix of the membrane.

In a previous paper (Flanagan & Forstner, 1978), we showed that the papain-solubilized enzyme was dissociated into five species of lower molecular weight by lowering the pH below 5.0 or boiling in the presence of SDS or in urea. The dissociated species were stable on electrophoresis, had a similar amino acid composition and were thought to represent stable aggregates of a common monomer. Since the pattern of dissociation might be substantially altered by the presence of hydrophobic peptides cleaved by papain, we undertook to purify maltase/glucoamylase after its solubilization by Triton X-100. In this paper we describe the purification of the detergent-solubilized enzyme to an extent comparable with that of the papain-solubilized enzyme and compare some of its properties, including that of dissociation, with those of the papain-solubilized enzyme.

Experimental

Chemicals and reagents

General reagents were of analytical grade from Fisher Scientific (Montreal, P.Q., Canada). Bovine serum albumin, glucose oxidase, horseradish peroxidase, 3,3'-dimethoxybenzidine (*o*-dianisidine) and Tris were obtained from Sigma (St. Louis, MO, U.S.A.), and sucrose, lactose, maltose, D-mannitol and ammonium persulphate were from Fisher Scientific (Montreal, P.Q., Canada). Acrylamide, *NN'*-methylenebisacrylamide and *NNN'N'*-tetramethylethylenediamine were products of Eastman (Rochester, NY, U.S.A.), and β -galactosidase was purchased from Worthington (Freehold, NJ, U.S.A.). Sepharose 4B and DEAE-cellulose (DE-52) were obtained from Pharmacia (Dorval, P.Q., Canada) and Whatman (Springfield Mill, Maidstone, Kent, U.K.) respectively. Tritiated L-amino acid mixture was obtained from New England Nuclear (Boston, MA, U.S.A.).

Animals

White male Wistar rats weighing 150–250 g were used throughout this study. The animals were starved for 12 h before death.

Enzyme assays

Maltase and sucrase activities were measured by the method of Dahlqvist (1968). Alkaline phosphatase was assayed by using *p*-nitrophenyl phosphate as described by Forstner *et al.* (1968). Leucine aminopeptidase activity was determined by the procedure of Goldberg & Rutenberg (1958) as modified by Forstner (1971). β -Galactosidase activity was measured with lactose as substrate

(Dahlqvist, 1968). A unit of enzyme activity represents 1 nmol of substrate hydrolysed/min.

Protein measurement

Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Gel filtration and ion-exchange chromatography

The conditions used for chromatography on Sepharose 4B and DEAE-cellulose are described in the text and in legends in the Results section.

Enzyme purification

Step 1: solubilization of maltase. White Wistar rats were killed by cervical dislocation. The whole small intestine was then gently removed and washed thoroughly with ice-cold 0.9% NaCl. All subsequent steps were carried out at 4°C. The cleaned intestine was cut longitudinally and scraped gently with a glass microscope slide to remove the mucosa. Combined mucosal scrapings from six to ten rats were homogenized with 10 vol. (mg/g of tissue) of 10 mM-potassium phosphate buffer, pH 7.0, for 20 s at top speed in a Waring blender. The crude homogenate was centrifuged at 35 000 *g* for 30 min at 4°C in a SS 34 rotor of a Sorvall RC 2B centrifuge. The supernatant was discarded and the pellet was homogenized for 2 min in an equal volume of 10 mM-potassium phosphate buffer, pH 7.0, containing 0.5% Triton X-100, with eight strokes of a Potter–Elvehjem homogenizer. The homogenate was centrifuged for 1 h at 105 000 *g* in a Spinco type 30 rotor. The supernatant was kept for subsequent purification of maltase, and the precipitate was re-extracted twice with 0.5% Triton in 10 mM-potassium phosphate buffer, pH 7.0, to ensure maximum solubilization of the enzyme. In preliminary experiments, solubilization from the centrifuged pellet of four brush-border membrane enzymes, namely maltase, sucrase, alkaline phosphatase and leucine aminopeptidase, was studied by using concentrations of Triton X-100 of 0.5–3.0%. Maximum release of maltase (approx. 65%) was obtained at 0.5% Triton X-100. Approx. 25% of sucrase, 10% of leucine aminopeptidase and 5% of alkaline phosphatase were released at this concentration. Although maximum release of sucrase was obtained at 0.5% Triton X-100, release of leucine aminopeptidase and alkaline phosphatase increased gradually as the Triton X-100 concentration was raised to 3%. To minimize release of other proteins, 0.5% Triton X-100 was chosen in subsequent experiments as the optimum detergent concentration for the solubilization of maltase from the intestinal membrane. The usefulness of Triton X-100 in membrane chemistry is well established,

but, owing to the non-ionic character of the molecule and its extremely low critical micelle concentration (Holloway & Katz, 1972), it is difficult to remove the detergent from the analytical system. Triton X-100 was removed by butan-1-ol extraction as described by Sasajima *et al.* (1975). Equal volumes of ice-cold butan-1-ol were added to the combined supernatants and the mixture was stirred for 15 min in the cold. A milky-white suspension was formed, which could be separated into three phases by centrifugation at 25000g for 20 min. The upper organic phase and the white precipitate at the interphase were devoid of maltase activity and were discarded. The lower aqueous phase containing the enzyme was dialysed for 24 h against three changes (each 2 litres) of water and then freeze-dried. At the end of the butan-1-ol extraction the enzyme activity was enriched 7-fold compared with the crude homogenate. A tracer experiment using ^3H -labelled Triton X-100 (New England Nuclear) showed that less than 0.1% of the total Triton X-100 added still remained after this procedure. The remainder disappeared during column chromatography.

Step 2: Sepharose 4B chromatography. The dialysed and reconstituted supernatant fraction obtained after butan-1-ol extraction was applied to a Sepharose 4B column (2.5 cm $\times$ 90 cm), which had been equilibrated with 10 mM-potassium phosphate, pH 7.0. Elution was carried out with the same solution at a rate of 10 ml/h. In order to discriminate between maltase and maltase activity associated with sucrase/isomaltase, 2.0 ml fractions were collected and analysed for maltase and sucrase activities. A typical elution profile (Fig. 1) shows four peaks

with maltase activity. Peaks I and IV also had sucrase activity, whereas peaks II and III were sucrase-free. Peak I was eluted in the void volume and probably contains aggregated maltase as well as sucrase. It was not investigated further. Peak II, although it had maltase activity, was insignificantly small and was also discarded. The majority of non-aggregated maltase was eluted under peak III. The eluted fractions in peak III with maltase/sucrase activity ratio of less than 5:1 were pooled for further purification.

Step 3: DEAE-cellulose (DE-52) chromatography. The combined fractions containing maltase activity were applied to a DEAE-cellulose column (1.5 cm $\times$ 20 cm) previously equilibrated with 10 mM-potassium phosphate, pH 7.0. A linear gradient (total volume 500 ml) of 10–70 mM-potassium phosphate, pH 7.0, was used to elute the adsorbed proteins from the column. A minor peak containing sucrase was discarded. The major maltase peak occurred at the same volume as the papain-solubilized enzyme (at about 40 mM-potassium phosphate) and contained no sucrase activity. It was pooled, dialysed against water, freeze-dried, and the residue was resuspended in water at a concentration of 1 mg/ml.

Polyacrylamide-gel electrophoresis

The SDS dissociating method of Fairbanks *et al.* (1971) as modified by Steck & Yu (1973) was used throughout. The gel was 3.32% (w/v) acrylamide with net cross-linkage of 3.61%. Proteins (20–50 μg) were dissolved in the electrophoresis buffer, then water and SDS were added to a final volume of 100 μl . Electrophoresis was performed in glass tubes (1.0 cm $\times$ 10 cm) with 4 mM-Tris/HCl buffer, pH 7.4, containing 25 mM-EDTA and 10 mM-sodium acetate at a current of 8 mA per tube for about 1½ h at room temperature until the Bromophenol Blue marker had migrated 9.0 cm. The dye front was located by stabbing the gel with a needle dipped in Indian ink. Protein on the gel was stained with Coomassie Blue and destained in 10% (v/v) acetic acid.

Density-gradient centrifugation

This was carried out by the method of Martin & Ames (1961) as modified by Forstner & Galand (1976). Continuous 5 ml linear gradients of 0.5–28% (w/v) D-mannitol in 100 mM-Tris/HCl buffer, pH 7.4, were made at room temperature with a Beckman gradient former and used within 1 h after preparation. Samples (0.01–0.1 ml) were layered on the gradient, and 1 unit ($\mu\text{mol}/\text{min}$) of β -galactosidase was then added as an internal molecular-weight marker (mol.wt. 540000; 16.6S). Centrifugation was carried out at 150000g at 4°C in an SW 50.1 swinging-bucket rotor for 5 h. Tubes were punctured at their base by a hollow needle and seven-drop

Fig. 1. Chromatography of Triton-solubilized maltase on Sepharose 4B

The column (2.5 cm $\times$ 90 cm) was equilibrated with 10 mM-potassium phosphate, pH 7.0, and the adsorbed enzyme was then eluted with the same solution. A flow rate of 10 ml/h was used, and fractions (2.5 ml) were collected and analysed for maltase (●) and sucrase (○) activities by the method of Dahlqvist (1968) as described in the Experimental section.

(0.1 ml) fractions collected for analysis. The enzymes of interest sedimented linearly with time in this gradient, and the rim of mannitol precipitation at the base of the tube did not affect the collection of the gradient.

Analysis of amino acid composition

Solutions of the pure enzyme were rendered free of salt by dialysis and then hydrolysed in 6M-HCl for 16 h at 100°C in evacuated tubes. Amino acid analyses were performed with a Beckman-Spinco automatic model 120 C amino acid analyser.

Immunodiffusion

Immunodiffusion was carried out by the double-diffusion technique of Ouchterlony (1968) modified by Forstner *et al.* (1973). Antisera to the detergent-solubilized and papain-solubilized maltases were prepared as described previously (Flanagan & Forstner, 1978).

Preparation of tritiated maltase

The intestinal mucosa was labelled by a modification of the method of Alpers (1972, 1977). Six male Wistar rats (120–150 g) were starved overnight. A tritiated mixture of 15 amino acids (0.15 mCi/rat) in 0.9% NaCl was injected into the upper jejunum of animals anaesthetized with ether. The entire intestinal lumen was filled with fluid (about 5.0 ml). The needle hole was sealed by application of Eastman 910 adhesive. The abdomen was then closed by suture and the animals were maintained on water for 5–6 h to allow incorporation of label into the intestinal mucosa before they were killed. The combined mucosal scrapings from the rats were used for the isolation and purification of maltase as described in the previous section.

Determination of N-terminal residues

The dansyl (5-dimethylaminonaphthalene-1-sulphonyl) chloride procedure first described by Hartley

(1970) was used with the following modifications. A portion (50 µg) of maltase in water was evaporated to dryness in a 0.3 ml mini-Reacti vial (Pierce Chemical Co., Rockford, IL, U.S.A.), in a vacuum desiccator over KOH pellets at room temperature. This was followed by the addition of 20 µl of 0.2 M-NaHCO₃, pH 9.8, and 100 µg of dansyl chloride in acetone. The mixture was incubated for 1 h at 37°C and then dried in vacuum. The dansylated derivatives were hydrolysed in 50 µl of 6M-HCl for 18 h at 100°C. HCl was removed by evaporation over KOH pellets. The hydrolysate was taken up in 20 µl of 50% (v/v) pyridine and characterized by two-dimensional chromatography on micro-polyamide sheets (5 cm × 5 cm) (Accurate Chemical and Scientific Corp., Hicksville, NY, U.S.A.). Samples (1.0 µl) of the hydrolysate were spotted at one corner of polyamide sheets, which were developed with 1.5% (v/v) formic acid in the first dimension and benzene/acetic acid (9:1, v/v) in the second dimension. The identities of dansylated derivatives from maltase were confirmed by chromatography of dansylated amino acid standards.

Results

Purification of maltase

Table 1 summarizes results obtained at several stages of purification in a single experiment. A 200-fold purification of maltase with 7–10% yield was achieved routinely. The final preparation of Triton X-100-solubilized maltase had a specific activity of 70.3 ± 7.1 units/mg of protein in six preparations, comparable with that previously published for the proteolytically solubilized maltase prepared in our laboratory (Flanagan & Forstner, 1978). In SDS/polyacrylamide-gel electrophoresis both the detergent-solubilized and papain-solubilized enzyme migrated as a single protein band with the same electrophoretic mobility ($R_F = 0.12$; Fig. 2). In a 0.5–28% D-mannitol gradient, both the Triton-solubilized and papain-solubilized maltase

Table 1. *Purification procedure for intestinal maltase from rats*
Enzyme activity was measured by the method of Dahlqvist (1968) (for full details see the text).

Fractions	Total maltase (units)	Total protein (mg)	Sp. activity (units/mg)	Purification factor	Yield (% recovery)
Crude homogenate	953	3800	0.3	1	100
35 000 g combined ppt.	808	1568	0.5	2.1	85
105 000 g supernatant (Triton)	775	1087*	—	—	—
Aqueous-butan-1-ol extract	790	412	1.9	7.7	83
Before Sepharose 4B chromatography	560	176	3.2	12.8	59
After Sepharose 4B chromatography	238	7	34.0	136.0	25
After DEAE-cellulose chromatography	67	1.3	51.5	206.0	7

* Owing to the interference of Triton X-100 with the protein determination, specific activity of the enzyme at this stage of purification was not accurate.

Fig. 2. SDS/polyacrylamide-gel electrophoresis of purified Triton-solubilized and papain-solubilized maltase

(a) Triton-solubilized maltase (50 µg of protein applied to gel); (b) papain-solubilized maltase (80 µg of protein applied to gel). Electrophoresis was performed as described in the Experimental section. The arrow on the left indicates the origin and the inked stab-marks at the bottom of the gels indicate the migration of the Bromophenol Blue marker.

co-sedimented with the internal molecular-weight marker, β -galactosidase (Fig. 3) after 5 h of centrifugation at 150 000g.

As shown in Table 2, the amino acid compositions of the two forms of maltase were not particularly different when analysed simultaneously. The sum of apolar amino acid residues (glycine, alanine, valine, methionine, isoleucine, leucine, phenylalanine) in the detergent-solubilized enzyme amounted to 37.1 residues/100 amino acids, as compared with 36.0 residues in the proteolytically digested enzyme. These results suggest that the detergent-solubilized enzyme does not have a great excess of apolar amino acids as compared with the proteolytically solubilized enzyme. Our previous results (Flanagan & Forstner, 1978) indicated that papain-solubilized enzyme had 41% apolar amino acids. The difference is largely attributable to the very low serine determination (2.0 mol% in comparison with 11.3 mol%). The earlier results were obtained after hydrolysis in 6.0M-HCl for 30 h,

Fig. 3. Density-gradient centrifugation of the detergent- and papain-solubilized maltases

Samples (0.01–0.1 ml) were layered on the 5 ml linear gradients of 0.5–28% D-mannitol in 100mM-Tris/HCl buffer, pH 7.4; 1 unit of β -galactosidase was then added as an internal molecular-weight marker, and the tubes were centrifuged for 5 h at 150 000g in a SW 50.1 swinging-bucket rotor as described in the Experimental section. Triton-solubilized maltase (●), papain-digested maltase (▲) and β -galactosidase (○) activities were assayed in each fraction (0.1 ml) collected from the bottom of the tube. Activity is expressed as a percentage of maximum and data are plotted as activity versus fraction number.

rather than 16 h as in the present study. As commented upon at that time, they were probably related to losses of the hydroxyamino acids during hydrolysis.

Immunodiffusion

Immunodiffusion studies were carried out with antisera prepared against both the detergent-solubilized and the proteolytically solubilized enzyme. The two forms of the enzyme gave single precipitin lines which fused completely with either antibody, indicating that neither had elicited antibody against an unshared antigenic component.

Table 2. *Amino acid composition of rat intestinal maltase/glucoamylase*

Enzyme (25 µg) was hydrolysed in 6M-HCl for 16h under vacuum for amino acid determination. The detergent-solubilized enzyme was analysed in triplicate (results are means $\pm$ S.E.M.), whereas the papain-solubilized enzyme was analysed once under the same conditions at the same time. Abbreviation: n.p., not present.

	Amino acid content (mol/100 mol)	
	Detergent-solubilized enzyme (Mean $\pm$ S.E.M.)	Papain-solubilized enzyme
Asp	12.7 $\pm$ 0.9	13.6
Thr	6.3 $\pm$ 0.3	5.4
Ser	11.8 $\pm$ 0.7	11.3
Glu	11.0 $\pm$ 0.6	14.7
Pro	6.0 $\pm$ 0.4	4.9
Gly	12.9 $\pm$ 2.2	12.3
Ala	6.4 $\pm$ 0.2	5.9
½Cys	n.p.	n.p.
Val	3.5 $\pm$ 0.7	2.8
Met	Trace	1.2
Ile	2.7 $\pm$ 0.5	3.0
Leu	6.3 $\pm$ 0.7	6.9
Tyr	6.1 $\pm$ 2.2	4.4
Phe	4.9 $\pm$ 0.8	3.9
Lys	3.7 $\pm$ 0.3	4.5
His	2.2 $\pm$ 0.2	1.9
Arg	2.8 $\pm$ 0.4	3.1
	99.3	100.0
Apolar amino acids	37.1	36.1

Triton X-100 binding

Amphipathic molecules isolated and purified in detergent solutions have shown a strong tendency to aggregate in aqueous solution or to maintain their solubility by strong and persistent interaction with detergent micelles even though transferred to aqueous solutions (Sigrist *et al.*, 1975; Hughey & Curthoys, 1976). Our detergent-solubilized maltase was eluted at the same volume from Sepharose 4B as the papain-solubilized enzyme and had the same sedimentation velocity in mannitol gradients, indicating that the enzyme was not in aggregated form. Several experiments were performed in an effort to determine whether the detergent-solubilized enzyme interacted strongly with detergent. In these experiments the binding of tritiated Triton X-100 was determined by ultracentrifugation of the enzyme plus labelled detergent on mannitol gradients as described for Fig. 4. Enzyme was mixed with the labelled detergent in tracer quantities, or in concentrations above the critical micellar concentration for Triton X-100 either at the initiation of the centrifugation run or before incubation for 16h at 4°C before the centrifugation run. Gradient conditions were also varied by adding variable amounts of unlabelled Triton X-100 to the gradients during their preparation. Sample results are shown in Fig. 4. There

was no evidence of binding of tritiated Triton X-100 to the enzyme under any condition, and at all times the migration of maltase within the gradient remained unchanged relative to β -galactosidase or proteolytically solubilized maltase markers. The molecular state of the maltase was not affected, therefore, by the presence of Triton X-100, and there was no evidence that the detergent was bound by the purified enzyme.

Aggregation of the 'aged' detergent-solubilized enzyme

In spite of its apparent lack of apolarity, solubility in monomeric form and lack of interaction with detergents, aggregation of the detergent-solubilized enzyme was noted in preparations that had been stored in distilled water at -20°C for several months. Aggregation occurred without loss of maltase activity and was either partial or complete depending on the age of the enzyme. The ability to aggregate under these conditions clearly differentiates the detergent-solubilized enzyme from the proteolytically solubilized form, since we have never observed aggregation with samples of the latter stored for as long as 2 years. Fig. 5 shows results obtained by passage of a 2-month-old preparation of the detergent-solubilized enzyme which had been label-

Fig. 4. Migration of Triton-solubilized maltase in mannitol gradients containing (b) 1% and (a) 5% Triton X-100

Linear 5 ml gradients were prepared from 0.5 and 28% mannitol dissolved in 100 mM-Tris-HCl buffer, pH 7.4, containing the indicated concentrations of Triton X-100. Then 50 μ g of the detergent-solubilized maltase (●), 1 unit of β -galactosidase (○) and trace amounts of 3 H-labelled Triton X-100 (■) were layered on each gradient and incubated overnight at 4°C with the enzyme. Centrifugation was carried out in an SW 50.1 rotor at 4°C for 5 h at 150 000 g. Fractions (0.1 ml) were collected from the bottom of each tube. Enzyme activities and radioactivity were then measured

led with tritiated amino acids through a column of Sepharose 4B. In contrast with the papain-solubilized enzyme, which was eluted between fractions 25 and 30, the labelled detergent-solubilized enzyme exhibited a second peak which was excluded from the column. This second peak was never seen in fresh preparations of the detergent-solubilized enzyme, or in papain-solubilized preparations. Fig. 6 illustrates the ultracentrifugal behaviour of a 3-month-old preparation of the detergent-solubilized enzyme on a mannitol gradient. On this occasion all of the maltase activity precipitated to the bottom of the tube, whereas a proteolytically solubilized enzyme, of identical age, migrated in the customary position. Evidence of aggregation was also observed on SDS/polyacryl-

Fig. 5. Chromatography of a mixture of tritiated-detergent-solubilized and papain-digested maltase on Sepharose 4B

The column (1 cm $\times$ 52 cm) was equilibrated and eluted with 10 mM-potassium phosphate, pH 7.0. Radioactivity and maltase activity were determined in the eluted fractions (1 ml). ○, Combined maltase activity, i.e. Triton-solubilized and papain-solubilized mixture; ■, 3 H-labelled-Triton-solubilized maltase; ▲, papain-solubilized maltase (location from second run).

amide-gel electrophoresis with aged detergent-solubilized preparations as a complete or partial failure to enter 3.3%-acrylamide gels. Pretreatment of the aggregated material with strong dissociating agents such as 100% mercaptoethanol, 8 M-urea or 5 M-guanidine hydrochloride failed to produce disaggregation.

Dissociation of the detergent-solubilized enzyme

We had previously shown that the papain-solubilized enzyme dissociated into five inactive species on SDS/polyacrylamide gel after the enzyme had been boiled at 100°C for 2–5 min in the presence of SDS (Flanagan & Forstner, 1978). Fig. 7 demonstrates that the detergent-solubilized enzyme is dissociated under these conditions into five species, which appear to have R_F values which are identical with those of the species from the papain-solubilized enzyme. Table 3 indicates that the amount of protein in each species is also identical in the two enzymes, as judged by staining density with Commassie Blue.

N-Terminal analysis

Two major N-terminal amino acids were found in both native enzymes, glycine and arginine. These two N-termini appeared concomitantly in each of the

Fig. 6. Effect of enzyme storage on the detergent- and papain-solubilized maltase in density-gradient centrifugation as described in the text

Centrifugation was performed in a SW 50.1 rotor for 5 h at 150 000g with β -galactosidase as an internal molecular-weight marker. (●) Triton-solubilized maltase and (▲) papain-digested maltase activity was determined in each 0.1 ml fraction collected from the bottom of the tube. Activity is expressed as a percentage of maximum and data are plotted as activity versus fraction number.

Table 3. Relative distribution of bound Coomassie Blue stain in the dissociated species from the detergent-solubilized and the papain-solubilized maltase in 3.3%-polyacrylamide gels

The procedures were carried out as described in the Experimental section. The gels, stained with Coomassie Blue, were scanned at 550 nm in a Gilford 2400 spectrophotometer and the areas under the appropriate peaks were measured by triangulation. The amount of bound stain in each species was expressed as a percentage of the total, $\pm$ S.E.M. from three experiments.

Species	Density of Coomassie Blue (% of Total)	
	Detergent-solubilized enzyme	Papain-solubilized enzyme
1	33.2 $\pm$ 0.2	36.7 $\pm$ 2.8
2	21.1 $\pm$ 3.9	23.7 $\pm$ 1.1
3	11.9 $\pm$ 2.9	13.3 $\pm$ 0.3
4	19.7 $\pm$ 1.7	18.4 $\pm$ 0.6
5	14.1 $\pm$ 0.9	7.9 $\pm$ 1.7

three larger species, 3, 4, and 5. The only *N*-terminus in species 1 was glycine, and in species 2, arginine. The *N*-termini were the same in the dissociated species from both the detergent-solubilized and the proteolytically solubilized maltase.

Fig. 7. SDS/polyacrylamide-gel electrophoresis of purified Triton-solubilized (a) and papain-solubilized (b) maltase after boiling for 2–5 min at 100°C

In each case 100 μ g of protein was applied to the gel. Electrophoresis was performed as described in the Experimental section. The arrow on the left indicates the origin and the ink marks at the bottom of the gels indicate the migration of the Bromophenol Blue mark.

Discussion

The present experiments were undertaken in the expectation that they would provide evidence of a specific terminal apolar segment in the detergent-solubilized form of rat maltase/glucoamylase which would account for its attachment to the intestinal brush-border membrane, and its ready liberation by proteinases such as papain. Maroux & Louvard (1976) have provided evidence of such a segment, by isolation of apolar peptides from a detergent-solubilized pig maltase/glucoamylase after digestion with trypsin. Our results are nevertheless remarkable in demonstrating the extreme similarity of the detergent-solubilized and proteolytically solubilized forms of maltase/glucoamylase. In contrast with experience with detergent-solubilized forms of other brush-border enzymes, sucrase/isomaltase (Sigrist *et al.*, 1975) and aminopeptidase (Louvard *et al.*, 1975; Maroux & Louvard, 1976), fresh preparations of the Triton X-100-solubilized

enzyme showed no tendency to aggregate in aqueous buffers. Also, in contrast with γ -glutamyl transpeptidase (EC 2.3.2.2) (Hughey & Curthoys, 1976), there was no evidence that the purified enzyme either bound detergent or required it for solubility.

During extraction, however, approx. 20% of the total maltase activity appeared as a high-molecular-weight species at the void volume of Sepharose 4B. Some of this activity is probably derived from the maltase associated with sucrase/isomaltase (Kolinska & Semenza, 1967; Gray *et al.*, 1979), but it is likely as well that it included a considerable proportion of maltase/glucoamylase. Thus some maltase/glucoamylase was probably extracted as an aggregate, and it could be argued that our experiments had selected for an altered enzyme that had lost an apolar component through cleavage by endogenous proteinases. The strongest evidence against this possibility is the demonstration that aggregation occurred when detergent-solubilized enzyme preparations were stored at -20°C for periods beyond 2 months. Aggregation has not been noted with the proteolytically solubilized enzyme up to a period of 2 years. Furthermore, as Figs. 5 and 6 demonstrate, aggregation, although apparently incomplete in some preparations, reached completeness in others, indicating that there were no molecules within the detergent-solubilized preparation that were incapable of aggregation with time. These findings show that the detergent-solubilized form of the enzyme is different from the form extracted with papain. Maltase/glucoamylase is released much more effectively by Triton X-100 than are other brush-border enzymes, suggesting that the apolar forces promoting membrane association must be relatively weak. Conformational changes after extraction may further weaken these forces by partially burying apolar binding sites within the molecule and thus preventing interaction with labelled detergent.

Other brush-border enzymes, aminopeptidase (Maroux & Louvard, 1976) and sucrase/isomaltase (Frank *et al.*, 1978), have been shown to possess unique *N*-terminal segments, which are found only in the detergent-extracted enzyme, and not after proteolytic digestion. These segments appear to be relatively apolar and presumably constitute the site of attachment of these enzymes to the intestinal plasma membrane. Both detergent-solubilized and proteolytically solubilized forms of rat maltase/glucoamylase have the same two *N*-termini, arginine and glycine, however, and the same two *N*-termini appear separately in the two smallest of the five species produced by boiling in detergent. Both enzymes therefore appear to be oligomers of two types of subunit. There is no evidence therefore that the detergent-solubilized form of maltase/glucoamylase has a unique apolar *N*-terminal segment. Our results do not suggest an alternative

explanation for the weak apolarity, which apparently accounts for the delayed aggregation of the detergent-solubilized enzyme. A C-terminal apolar segment is possible. In terms of electrophoretic mobility in SDS/polyacrylamide-gel electrophoresis the two putative monomers appear to have the same molecular weights in both enzymes, but they are of a size (80 000 daltons minimum, 245 000 daltons maximum; Flanagan & Forstner, 1978) where an additional apolar peptide segment would not be detected easily. One must also consider the possibility that maltase/glucoamylase binds to the plasma membrane by mechanisms which involve conformational uncovering of apolar segments within monomeric peptides, or perhaps carbohydrate interaction as in some lysosomal enzymes (Hickman & Neufeld, 1972; Neufeld *et al.*, 1975; Kaplan *et al.*, 1977), thus obviating the necessity of an apolar terminus.

If species 1 and 2 are the monomeric species of maltase/glucoamylase, species 3 may be a dimer made up of one unit of each. This conclusion is supported by previous molecular-weight estimates, which have assigned a molecular weight of 135 000 for species 1, 245 000 for species 2, and 350 000 for species 3. It is not possible, however, to arrive at a satisfactory prediction for the oligomeric composition of the native enzyme. Maltase/glucoamylase has previously been estimated to have molecular weight of approx. 500 000 (Flanagan & Forstner, 1978), which is compatible with trimeric structures of either the AAB or ABB type. The major species of maltase behave anomalously, however, on SDS/polyacrylamide-gel electrophoresis, effectively preventing accurate assessment of molecular weight (Flanagan & Forstner, 1978), and for this reason it is possible that a tetrameric structure, AABB, AAAB or even ABBB, would also be compatible with the available evidence.

This was supported by the Medical Research Council of Canada.

References

- Alpers, D. H. (1972) *J. Clin. Invest.* **51**, 167–173
- Alpers, D. H. (1977) *Biochem. Biophys. Res. Commun.* **75**, 130–135
- Alpers, D. H. & Solin, M. (1970) *Gastroenterology* **58**, 833–842
- Auricchio, S., Dahlqvist, A. & Semenza, G. (1963) *Biochim. Biophys. Acta* **96**, 498–507
- Dahlqvist, A. (1968) *Anal. Biochem.* **22**, 99–107
- Dahlqvist, A. & Thomson, D. L. (1963) *Biochem. J.* **89**, 272–277
- Eichholz, A. (1968) *Biochim. Biophys. Acta* **163**, 101–107
- Eichholz, A. & Crane, R. K. (1965) *J. Cell Biol.* **26**, 687–691
- Fairbanks, G., Steck, T. L. & Wallach, D. F. H. (1971) *Biochemistry* **10**, 2606–2617

- Flanagan, P. R. & Forstner, G. G. (1978) *Biochem. J.* **173**, 553–563
- Flanagan, P. R. & Forstner, G. G. (1979) *Biochem. J.* **177**, 487–492
- Forstner, G. G. (1971) *Biochem. J.* **121**, 781–789
- Forstner, G. G. & Galand, G. (1976) *Can. J. Biochem.* **54**, 224–232
- Forstner, G. G., Sabesin, S. M. & Isselbacher, K. J. (1968) *Biochem. J.* **106**, 381–390
- Forstner, J., Taichman, N., Kalnins, V. & Forstner, G. (1973) *J. Cell Sci.* **12**, 585–602
- Frank, G., Brunner, J., Hauser, H., Wacker, H., Semenza, G. & Zuber, H. (1978) *FEBS Lett.* **96**, 183–187
- Goldbarg, J. A. & Rutenberg, A. M. (1958) *Cancer (Philadelphia)* **11**, 283–291
- Gray, G. M., Lally, B. C. & Conklin, K. A. (1979) *J. Biol. Chem.* **254**, 6038–6043
- Hartley, B. S. (1970) *Biochem. J.* **119**, 805–822
- Hickman, S. & Neufeld, E. F. (1972) *Biochem. Biophys. Res. Commun.* **49**, 992–999
- Holloway, P. W. & Katz, J. T. (1972) *Biochemistry* **11**, 3689–3695
- Hughey, R. P. & Curthoys, N. P. (1976) *J. Biol. Chem.* **251**, 7863–7870
- Kaplan, A., Achord, D. & Sly, W. S. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 2026–2030
- Kelly, J. J. & Alpers, D. H. (1973) *Biochim. Biophys. Acta* **315**, 113–120
- Kolinska, J. & Kraml, J. (1972) *Biochim. Biophys. Acta* **284**, 235–247
- Kolinska, J. & Semenza, G. (1967) *Biochim. Biophys. Acta* **146**, 181–195
- Louvard, D., Maroux, S., Vannier, C. & Desnuelle, P. (1975) *Biochim. Biophys. Acta* **375**, 236–248
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Maroux, S. & Louvard, D. (1976) *Biochim. Biophys. Acta* **419**, 189–195
- Maroux, S., Louvard, D. & Baratti, J. (1973) *Biochim. Biophys. Acta* **321**, 282–295
- Martin, R. G. & Ames, B. N. (1961) *J. Biol. Chem.* **236**, 1372–1379
- Miller, D. & Crane, R. K. (1961) *Biochim. Biophys. Acta* **52**, 293–298
- Neufeld, E. F., Lim, T. W. & Shapiro, L. J. (1975) *Annu. Rev. Biochem.* **44**, 357–376
- Ouchterlony, O. (1968) *Handbook of Immunodiffusion and Immuno-electrophoresis*, Ann Arbor Science Publishers, Ann Arbor, MI
- Sasajima, K., Kawachi, T., Sato, S. & Sugimura, T. (1975) *Biochim. Biophys. Acta* **403**, 139–146
- Schlegel-Haueter, S., Hore, P., Kerry, K. R. & Semenza, G. (1972) *Biochim. Biophys. Acta* **258**, 506–519
- Seetharam, B., Swaminathan, N. & Radhakrishnan, A. N. (1970) *Biochem. J.* **117**, 939–946
- Sigrist, H., Ronner, P. & Semenza, G. (1975) *Biochim. Biophys. Acta* **406**, 433–446
- Sivakami, S. & Radhakrishnan, A. N. (1973) *Indian J. Biochem.* **10**, 283–284
- Steck, T. L. & Yu, J. (1973) *J. Supramol. Struct.* **1**, 220–232