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ON THE HYDROPHOBIC PART OF AMINOPEPTIDASE AND MALTASES WHICH BIND THE ENZYME TO THE INTESTINAL BRUSH BORDER MEMBRANE

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SUMMARY

The intestinal brush border aminopeptidase and unfractionated maltases M_2+M_3 are composed of a hydrophilic, sugar containing and enzymatically active part, and a smaller hydrophobic part presumably binding the catalytic part to the lipid matrix of the membrane Hydrophobic parts detached by trypsin from the detergent forms of aminopeptidase and the maltases were purified and shown to have molecular weights ranging from 8000 to 10000 All are rich in hydrophobic residues and contain no disulfide bridges. However, their overall amino acid composition is different The hydrophobic parts appear to be N-terminal in the detergent forms of the enzymes

INTRODUCTION

The neutral detergent Triton X-100 has recently been shown to solubilize the intestinal brush border hydrolases in an easily aggregating, amphipathic form which differs from the form liberated by papain [1, 2] Both are enzymatically active and the first is converted into the second by incubation with papain or trypsin [1, 2] They will be designated "detergent form" and "trypsin form"

Considering that aminopeptidase almost entirely emerges from the external side of the membrane bilayer [3], the above findings suggest that, like other membrane surface proteins (glycophorin [4], cytochrome b_5 [5], cytochrome b_5 reductase [6] and the 2 proteins of the SF virus membrane [7]), the brush border hydrolases are composed of a hydrophilic part bearing the function and a hydrophobic part insuring the fixation to the lipid matrix Detergents would liberate the entire molecule whereas papain on the intact mucosa and trypsin on the detergent form would liberate the hydrophilic part only, by cleaving strategically located peptide bonds

The hydrophilic part of porcine aminopeptidase has already been purified to homogeneity and found to contain 3 subunits with a total molecular weight of about 280 000 [1] The purpose of the present work is to show that the hydrophobic

parts of aminopeptidase and the mixture of maltases M_2 and M_3 [2] are relatively short polypeptide chains (molecular weight, about 9000) rich in apolar residues and containing no disulfide bridges

METHODS

Already published methods were used for the preparation of purified vesicles from porcine jejunum brush border membrane [8] and for enzymatic activity determinations [1 2] N-terminal residues were identified by a modified version of the dansylation technique [9] A C-terminal lysine was detected in one of the peptides by digestion with pancreatic carboxypeptidase B Trypsin (Worthington 2 crystallized) was bound to CNBr-activated Sepharose 4-B [10] Iodination with ^{125}I-labelled iodine (Amersham, > 14 Ci/mg) was carried out with the aid of lactoperoxydase [11] Emulphogen BC-720 was purchased from G A F (France)

RESULTS

Purification of the detergent forms of aminopeptidase and maltase

The detergent form of aminopeptidase was prepared by a procedure derived from that already published for the trypsin form [1] Aggregation was prevented by adding emulphogen BC 720 to all solutions Fresh jejunal mucosa (250 g) was homogenized under magnetic stirring for 1 h in twice its weight of a 10 mM phosphate buffer pH 6 0 After centrifugation, the pellet was resuspended in the same volume of buffer Emulphogen BC 720, a good substitute for Triton X-100 with no absorption at 280 nm, was added (2 5 %) and the mixture was stirred overnight at 4 °C Aminopeptidase was precipitated by ammonium sulfate between 0 3–0 6 saturation and passed through a column (3 × 250 cm) of Indubiose AC 3/4 (Industrie Biologique Francaise) in the phosphate buffer made 0 15 M in NaCl and 1 % in Emulphogen BC 720 The detergent form of the enzyme was eluted at exactly the same position as the trypsin form in the absence of detergent (1 6 V_0) After a 18-h dialysis against 10 l of the buffer 1 % in Emulphogen, the material was charged into a DEAE-cellulose column (2 × 10 cm) equilibrated and washed with the same buffer. Elution was performed with an NaCl concentration gradient from 0 to 0 3 M Fractions eluted between 0 115 and 0 140 M NaCl with specific activities higher than 20 000 units/mg were pooled, dialyzed as above and concentrated in a short DEAE-cellulose column (2 × 5 cm) eluted by the buffer 0 15 M in NaCl and 0 15 % in Emulphogen BC 720 The purification was 350-fold with a 25 % yield The final preparations had a specific activity similar to that of the pure trypsin form [1] They gave a single band (R_F, 0 100) by gel electrophoresis in the presence of 0 15 % of Emulphogen BC 720 A single band was also obtained after tryptic attack (R_F, 0 300) By contrast, 3 bands were obtained in 0 1 % sodium dodecyl sulfate, showing that the 3 subunits identified in the trypsin form pre-exist in the detergent form

For the maltases, a suspension of purified membrane vesicles (440 mg of protein) in the phosphate buffer was extracted as above by 2 5 % of Emulphogen BC 720 and the extract was chromatographed in a DEAE-cellulose column (3 10 cm) equilibrated with the buffer 1 0 % in Emulphogen BC 720 Sucrase and alkaline phosphatase were washed out by the buffer and the bulk of maltase activity was

separated from aminopeptidase and other contaminants by a linear NaCl gradient from 0 to 0 3 M Fractions with a constant specific activity (35 600 units/mg) were dialyzed and concentrated as above (purification, 10-fold, yield, 47 %) The final samples gave a single band (R_F, 0 130) by gel electrophoresis But, 2 bands (R_F, 0 410 and 0 730) were observed after tryptic treatment, corresponding to the already known maltases M_2 and M_3 All attempts to separate the detergent forms of these enzymes were unsuccessful

Purification of the hydrophobic parts

The same technique was used for the purification of the aminopeptidase and maltase parts The detergent forms were labelled with ^{125}I to facilitate subsequent detection of the split products and 5–6 mg/ml solutions in a 0 1 M Tris HCl buffer pH 7–8 were digested for 4 h at 4 °C by 0 5 mg/ml of Sepharose-bound trypsin After removal of the enzyme, the hydrolyzate was filtered through Sephadex G-50 giving the results shown by Fig 1 The material under peak I was further chromatographed on DEAE-cellulose as indicated in Fig 2 The retarded peak in this latter figure contained the hydrophilic trypsin form of the enzymes By contrast, the material under the excluded peak (no detectable absorption at 280 nm) was extractible by the Folch-Lees procedure [12] and hence could be considered strongly hydrophobic The yields calculated on a molar basis from the detergent form were 24 % for the aminopeptidase peptide (from the amino acid analysis) and 32 % for the trypsin-form of the enzyme

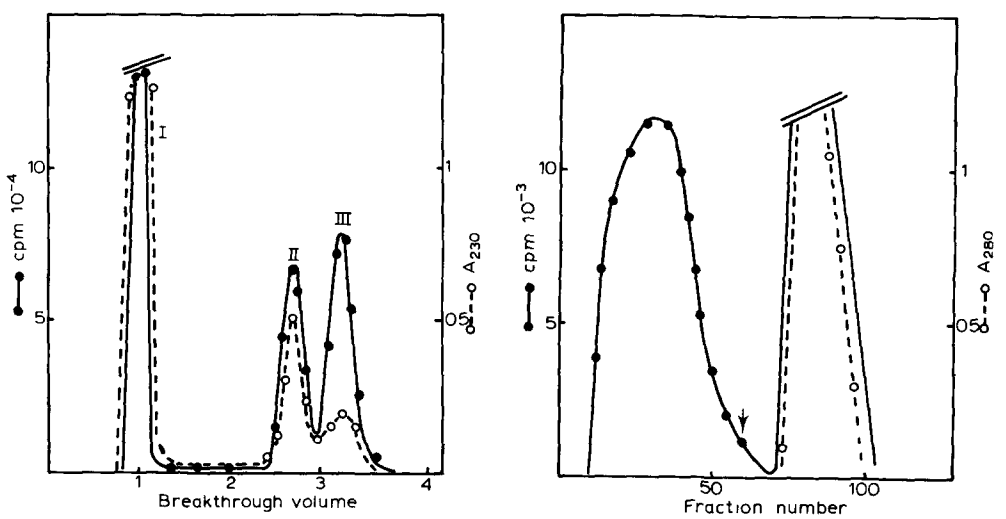


Fig 1 Sephadex G-50 filtration of the detergent form of aminopeptidase after labelling and tryptic cleavage The column (1 5 × 60 cm) was equilibrated and eluted with the 10 mM phosphate buffer 0 15 % in emulphogen BC 720 Peak III (10 % of the radioactivity) was free iodine Peak II (10 %), probably composed of several short peptides, was not further investigated See Fig 2 for subsequent fractionation of peak I (80 %)

Fig 2 DEAE cellulose chromatography of the material under peak I in Fig 1 The column (1 × 5 cm) was equilibrated and washed with the phosphate buffer, pH 6, 0 15 % emulphogen BC 720 After the emergence of the unretarded peak (arrow), a 2nd peak was eluted by 0 15 M NaCl in the buffer Volume fraction, 1 ml

Partial characterization of the hydrophobic parts

Fig 3a shows that the aminopeptidase hydrophobic part yielded by gel electrophoresis in the presence of sodium dodecyl sulfate, a single band which was found at the same position by radioactivity counting and staining by Coomassie Blue (M_r about 9000) In the case of the maltases (Fig 3b), 2 incompletely separated bands (M_r 10000 and 8300 respectively) were observed These bands probably correspond to the 2 hydrophobic parts yielded by maltases M_2 and M_3

The amino acid composition of the pure aminopeptidase hydrophobic part is given in Table I This hydrophobic part is seen to contain no disulfide bridge and a high number of hydrophobic residues (hence the low polarity index (35 %) typical of "intrinsic" membrane proteins [15]) Its minimum molecular weight calculated by summation of the residues is 8500 (9000 by gel electrophoresis (see above)) The results concerning the maltase hydrophobic parts are less significant However, they show as above that they are devoid of disulfide bridges and have the same very low polarity index Despite these common features, their overall amino acid composition is different

The N-terminal residues identified in the detergent and trypsin forms of the enzymes as well as in the corresponding hydrophobic parts are listed in Table II The presence of 2 N-terminal alanines in the detergent form of aminopeptidase and of 2 N-terminal aspartic acids in the trypsin form of the maltases is discussed below

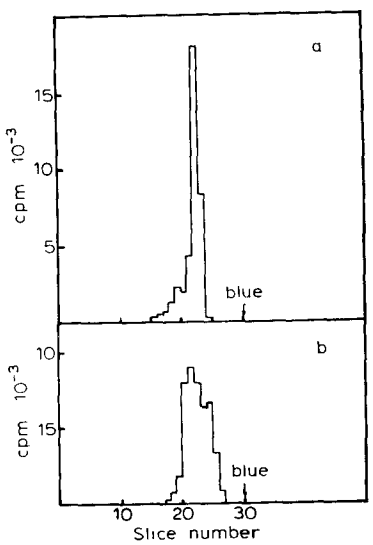


Fig 3 Electrophoresis on 15 % polyacrylamide gels in 0 1 % dodecyl sulfate [13] of the hydrophobic parts of aminopeptidase (a) and maltases (b) after a 15 min incubation at 70 C in 1 % sodium dodecyl sulfate and 1 % mercaptoethanol The position of the bands was ascertained by radioactivity counting in 2 mm thick slices The gels were calibrated with reduced-carboxymethylated bovine chymotrypsinogen A (M_r, 25 000), B and C chains of bovine α-chymotrypsin (13 000 and 11 000) and porcine colipase I (9100)

TABLE I

AMINO ACID COMPOSITION OF THE HYDROPHOBIC PEPTIDES

The molecular weight was calculated according to Delaage [14] in the case of the aminopeptidase peptide The same value was arbitrarily adopted for the 2 unfractionated peptides originating from maltases M_2 and M_3

	Peptides originating from			
	Aminopeptidase		Maltase M_2+M_3	
	Observed values*	Next integer	Observed values*	Next integer
Ala	11 16	11	5 29	5
Arg	1 03	1	3 10	3
Asx	3 05	3	4 82	5
Cys	0	0	0	0
Glx	5 10	5	5 95	6
Gly	8 65	9	4 53	4–5
His	1 3	1	0 55	0–1
Ile	8 81	9	4 05	4
Leu	10 36	10	15 68	16
Lys	4 72	5	4 07	4
Met	0 30	0–1	1 20	1
Phe	3 36	3	6 40	6
Pro	1 77	2	2 94	3
Ser	5 25	5	5 87	6
Thr	3 09	3	5 87	6
Tyr	4 30	4	1 40	1–2
Val	8 72	9	7 76	8
Total number of residues	–	80**	–	79–81**
Total weight	–	8460**	–	–
Polarity index***	–	35	–	35

* Average of the results obtained after 24 and 72 h hydrolysis at 115 °C in 5 7 M triple distilled HCl For Ser, Thr and Tyr, the value after extrapolation to zero time of the results obtained after 15, 24 and 72 h hydrolysis was taken For Leu and Ile, the highest value after 72 h hydroylsis was taken

** Tryptophan not included

*** Polarity index calculated according to Capaldi and Vanderkooi [15]

TABLE II

N-TERMINAL RESIDUES IN ENZYMES AND PEPTIDES

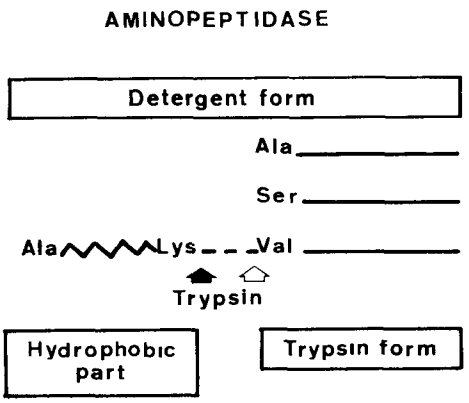
Aminopeptidase			Maltases M_2+M_3		
Detergent form	Trypsin form	Hydrophobic part	Detergent form	Trypsin form	Hydrophobic part
Ala	Ala	–	Ala	Ala	–
Ser	Ser	–	Leu	Asp	Phe
Ala	Val	Ala	Lys	Asp	Lys

DISCUSSION

The above results clearly show that 3 porcine intestinal brush border hydrolases (aminopeptidase, maltases M_2 and M_3), and probably also other hydrolases bound to the membrane are amphipathic molecules The sugar-containing, enzymatically active and bulky hydrophilic part almost entirely emerges from the external side of the membrane [3] The much smaller hydrophobic part identified in this work probably anchors the catalytic part to the bilayer Hence, this class of enzymes represents strongly oriented structures at the surface of the membrane The catalytic parts are directed towards the substrate-containing intestinal lumen whereas the hydrophobic parts are buried in the bilayer It is not yet known whether the fragments which are here designated hydrophobic parts are entirely buried or span the membrane It is also noteworthy that the size of the hydrophobic anchor is not proportional by far to that of the bound hydrophilic part (see for instance aminopeptidase and cytochrome b_5 [1–5]) This size might be a structural requirement for the integration in the biomembranes which all have similar thickness (45–110Å)

Finally, Table II shows that the trypsin form of aminopeptidase contains 3 N-terminal residues, in agreement with the result of 3 subunits already reported Therefore, the detergent form may be assumed to possess 2 N-terminal alanines, one leaving the molecule with the corresponding hydrophobic part and being replaced by a valine This mechanism, which implies that the hydrophobic part is N-terminal in one of the subunits of the enzyme molecule, is illustrated by Scheme I A sequence indicated by dots may exist between Lys and Val yielding the small peptides identified in peak II (Fig 1)

The same mechanism may be postulated for the maltases As expected, two N-terminal residues Phe and Lys, presumably related to the two reported hydrophobic peptides are identified To these peptides would correspond two N-terminal residue replacements, one of a lysine by an aspartic acid and the other of a leucine by an aspartic acid followed by a second tryptic cleavage yielding a N-terminal phenylalanine in the final peptide These postulations will require confirmation



Scheme I

when the structure of maltases M_2 and M_3 are known in more detail.

It has been postulated that aminopeptidase and disaccharidases might be involved in intestinal transport of amino acids [1, 16] and glucose [17] According to this hypothesis, we might assume that the hydrophobic parts play not only a role in the binding of these hydrolases but also participate in the transport processes.

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