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Subunit structure of pig small-intestinal brush-border aminopeptidase N

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Aminopeptidase N (EC 3.4.11.2), when isolated from pig intestine in either the proteinase- or detergent-released form, frequently appears to contain three polypeptide chains, here termed α, β and γ. We have established by an immunological technique that the β- and γ-polypeptides are derived from the α-chain and that the intact enzyme is a dimer, α_2. Each α-chain of the detergent form was shown to contain a hydrophobic anchor peptide about 35 amino acid residues in length, which included the *N*-terminal sequence. A peptide bond in the α-chain was very sensitive to proteolysis. Its cleavage generated the commonly observed forms: $\alpha\beta\gamma$ and $\beta_2\gamma_2$. The γ-fragment, which lacked the anchor peptide, was derived from the *C*-terminal part of the α-chain.

The small-intestinal brush-border membrane contains two aminopeptidases, designated N and A (EC 3.4.11.2 and EC 3.4.11.7), with a marked specificity for, respectively, neutral and acidic *N*-terminal amino acids in peptides and synthetic substrates. Whereas the rabbit enzymes are monomeric after solubilization from the membrane (Feracci & Maroux, 1980; Gorvel *et al.*, 1980), both aminopeptidases N and A purified from pig intestine are composed of several subunits or polypeptide chains (Maroux *et al.*, 1973; Sjöström *et al.*, 1978; Benajiba & Maroux, 1980). Aminopeptidase A from this source is a symmetrical dimer, with each of the two chains possessing a hydrophobic anchor peptide holding them at the membrane surface (Benajiba & Maroux, 1980). Aminopeptidase N was considered at first to contain three polypeptide chains of different size (molecular weights 130000, 97000 and 49000), at least one of them being anchored in the membrane by its *N*-terminal hydrophobic sequence (Maroux *et al.*, 1973; Maroux & Louvard, 1976). These three polypeptide chains were originally named A, B and C (Sjöström *et al.*, 1978; Norén & Sjöström, 1980), but α, β and γ by Svensson (1979) and in the present work. Sjöström *et al.* (1978) showed that pig intestinal aminopeptidase N was a dimer, which could be fragmented by a limited proteolysis *in situ* or during purification. But it was not known if this proteolysis occurred at random or was a process specific for one of the two polypeptide chains, which were judged to be identical in molecular weight (Norén & Sjöström, 1980). These authors also showed the A- and B-polypeptides were labelled by a lipid-soluble photosensitive reagent, suggesting that each carried an anchoring peptide. However, the presence of two anchor peptides in the original dimer could not be proved, owing to the qualitative character of the technique used.

In the present work we demonstrate that aminopeptidase N, like aminopeptidase A, is a symmetrical molecule anchored in the membrane by two hydrophobic peptides, corresponding to the *N*-terminal sequence of the two identical α-chains of the intact enzyme. These two chains may be converted into β- and γ-fragments, such that the anchor peptide is retained in the β-polypeptide.

Abbreviations used: abbreviations for amino acids follow IUPAC–IUB recommendations [see *Biochem. J.* (1972) **126**, 773–780]; SDS, sodium dodecyl sulphate; aminopeptidase d-form and p-form, enzymically active forms solubilized from the membranes by treatment with neutral detergent or by proteolytic digestion respectively.

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Experimental

Materials

DEAE-cellulose (microgranular grade DE-32) was obtained from Whatman (Maidstone, Kent, U.K.), Sepharose 4B, octyl-Sepahrose CL-4B and phenyl-Sepharose CL-4B were from Pharmacia (Uppsala, Sweden) and Ultrogel AcA-34 and concanavalin A–Indubiose was from Industrie Biologique Française (Villeneuve–La Garenne, France). The substrate L-leucine *p*-nitroanilide was purchased from Bachem (Marina de Prey, CA, U.S.A.).

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D-Amino acid oxidase from pig kidney, L-amino acid oxidase from *Crotalus adamanteus* venom and crystalline bovine liver catalase were obtained from Worthington (Freehold, NJ, U.S.A.). FAD, D- and L-amino acid kits and 3,5-di-iodosalicylic acid (lithium salt) were from Sigma (St. Louis, Mo, U.S.A.). Emulphogen BC 720 (a non-ionic detergent) was from GAF (Le Louvres, France).

Gel electrophoresis in SDS

Analytical electrophoresis (40 mA for 3 h) was performed in Fairbanks buffer (Fairbanks *et al.*, 1971) in 0.7 mm-thick polyacrylamide-gel slabs. The separating gel (8 cm; 5.6% acrylamide) and the stacking gel (2 cm; 5.0% acrylamide) were prepared as described by Fairbanks *et al.* (1971) and Laemmli (1970) respectively. After protein staining with Coomassie Blue, the gels were transferred on to a Whatman 3MM paper sheet and dried under vacuum in a slab dryer at 60°C for 30 min. Material labelled with ^{125}I by the lactoperoxidase method (Pagès *et al.*, 1975) was located by radioautography with Kodak X S-5 Omat S X-ray films.

Preparative electrophoresis was performed under similar conditions in 3 mm-thick slabs with a current of 100 mA. Samples (100 μl) containing 0.8 mg of reduced and carboxymethylated ^{125}I-labelled aminopeptidase (Crestfield *et al.*, 1963) were deposited in each well of the preparative gels. For location of protein bands, a 2–3 mm-wide longitudinal guide-strip was cut off from the slab, frozen and sliced into 2 mm sections with a Joyce–Loebl gel slicer, and the radioactivity was measured in each slice. The protein-containing slices were fragmented, mashed and stirred for 20 h at room temperature in 5 ml of 50 mM-sodium phosphate buffer, pH 8.2, containing 0.1% SDS. After elimination of the gel fragments by low-speed centrifugation, the supernatants contained 80% of the initial radioactivity.

Crossed immunoelectrophoresis

Electrophoresis in the first dimension was performed as described above in 1.4 mm-thick slabs. Longitudinal strips containing the separated polypeptide chains were cut off at once and inserted for the second dimension into 1.7 mm-thick plates (10 cm × 10 cm) of 1% agarose containing 1% Emulphogen and 500 μg of immunoglobulins from sera of guinea pigs immunized against the α-chain of p-aminopeptidase or against the mixture of the three polypeptide chains resulting from dissociation of the complex. The agarose-gel buffer as well as the conditions used for immunization of guinea pigs, preparation of immunoglobulins, immunoelectrophoresis and protein staining have been described previously (Feracci & Maroux, 1980). Immunoprecipitates of ^{125}I-labelled material were located by radioautography.

Assay of enzyme activity and determination of protein

Aminopeptidase N activity was measured spectrophotometrically at 410 nm, with L-leucine *α-p*-nitroanilide as substrate (Benajiba & Maroux, 1980). One unit is defined as the amount of enzyme liberating 1 μmol of *p*-nitroaniline/min under the conditions of the assays. Specific activities (units/mg of protein) were calculated by using for the protein the absorption coefficient ($A_{1\,cm}^{1\%} = 15.6$ at 280 nm) purified aminopeptidase (Maroux *et al.*, 1973).

Identification of N-terminal residues

This was performed by the dansylation method as previously described (Maroux & Louvard, 1976).

Purification of the d- and p-forms of pig aminopeptidase N

The earlier method of purification of both the p- and d-forms of pig aminopeptidase N (Maroux *et al.*, 1973; Maroux & Louvard, 1976) yielded a preparation that was contaminated by small amounts of aminopeptidase A as well as by an unidentified, strongly antigenic, component derived from goblet cells (A. Bernadac, A. Benajiba & S. Maroux, unpublished work).

The first steps of the new procedure up to the concanavalin A–Indubiose chromatography were the same as those described in detail for the purification of pig intestinal aminopeptidase A (Benajiba & Maroux, 1980). Full purification of the p-form of aminopeptidase N was achieved by passing the concanavalin A–Indubiose eluate through a column (2 cm × 25 cm) of DEAE-cellulose equilibrated and washed with 10 mM-sodium phosphate buffer, pH 6.0. The enzyme was eluted by a linear NaCl gradient (2 × 250 ml, from 0 to 300 mM) in the salt concentration range 100–140 mM. The fractions (specific activity 23 units/mg) were pooled and concentrated as previously described (Benajiba & Maroux, 1980) by passage through a short DEAE-cellulose column.

As previously described for rabbit aminopeptidase N (Feracci & Maroux, 1980) and pig aminopeptidase A (Benajiba & Maroux, 1980), a step involving the use of anti-(impurities) immunoabsorbent chromatography was necessary for full purification of the d-form of pig aminopeptidase N. The column was prepared as described previously (Benajiba & Maroux, 1980), with, for the preparation of the impurities fraction, immunoglobulins raised against the p-enzyme form. Active enzyme fractions migrating unretarded through the column were chromatographed on DEAE-cellulose as described above for the p-form, with buffers containing 1% Emulphogen and 1 mM-benzamidine. The Emulphogen concentration was lowered to

0.1% during the second passage through DEAE-cellulose. The specific activity of the final solutions was the same as that previously reported for the p-form (23 units/mg). No contamination was detected in either form by gel electrophoresis and crossed immunoelectrophoresis. The complete absence of p-form from the d-form preparations was also checked by these techniques (Louvard *et al.*, 1975; Benajiba & Maroux, 1980).

Separation of the d- and p-forms by hydrophobic chromatography

As already reported (Benajiba & Maroux, 1980), the amphiphilic d-form could easily be separated from the hydrophilic p-form by using phenyl-Sepharose or octyl-Sepharose columns equilibrated with 10mM-sodium phosphate buffer, pH 6.8, at room temperature. Under these conditions, the p-form migrated unretarded, whereas the d-form was bound. One ml of the hydrophobic matrix was found to bind 2mg of the d-form dissolved in a maximal volume of 3ml of the buffer containing 0.1% Emulphogen. The d-form was eluted later by washing the column with a buffer containing at least 2% Emulphogen or 0.2% SDS.

Isolation of the anchor peptide

Anchor peptide was prepared from trypsin hydrolysate of purified d-aminopeptidase as previously described (Maroux & Louvard, 1976).

Determination of the molecular weight of the anchor peptide by the isotopic-dilution technique

This technique has been already described in detail in the case of rabbit aminopeptidase N (Feracci & Maroux, 1980) and of pig aminopeptidase A (Benajiba & Maroux, 1980).

Amino acids of the anchor peptide

The amino acid composition of the peptide was determined with the aid of a Beckman model 120C amino acid analyser after a 24h hydrolysis in 6M-HCl at 115°C.

The optical configuration of these amino acids was determined by an enzymic method (Meister & Wellner, 1963). Dried amino acid samples originating from 12nmol of the peptide were dissolved in 300μl of 0.2M-Tris/HCl buffer, pH 8.0, and incubated, either with 5μl of L-amino acid oxidase (6.5mg/ml) in the presence of 3μl of catalase (1mg/ml), or with 20μl of D-amino acid oxidase (1mg/ml) in the presence of 3μl of catalase and 2μl of FAD (2mg/ml). After 24h, the same amount of enzyme was added and incubation was continued for a further 24h. Then, the reaction was stopped by 0.5ml of 2M-citrate buffer, pH 2.0, and the unmodified amino acids were determined as above. Under the conditions employed, the L-amino acid oxidase deaminated L-isoleucine, L-leucine, L-methionine, L-tyrosine, L-phenylalanine, L-arginine and L-valine with a 100% yield. The yield was 70% with L-alanine and L-lysine, and 20–30% with L-proline, L-aspartic acid, L-glutamic acid, L-serine, L-threonine and glycine. With the D-amino acid oxidase, the deamination yield was 100% for D-aspartic acid, D-alanine, D-valine, D-methionine, D-isoleucine, D-leucine, D-tyrosine and D-phenylalanine, 60% for D-serine and 20% for D-threonine and D-glutamic acid. Both enzymes were strictly specific for the L- or D-configuration.

Results

Preparation of the polypeptide chain and proteolytic fragments

Full dissociation of the peptides was obtained by reduction and carboxymethylation of the purified p- and d-aminopeptidase in 1% SDS. The peptides originating from the p-form of the enzyme are referred to below as p-α-chain and p-β- and p-γ-polypeptides, and those prepared from the d-form as d-α-chain and d-β- and d-γ-polypeptides.

The gel-electrophoresis patterns given by the dissociated p- and d-forms and also by each fragment already resolved by a first electrophoresis run are reproduced in Fig. 1. With both forms, three well-separated bands were obtained. However, the preparation by electrophoresis of sufficient amounts of the least abundant p-α- and d-α-chains was

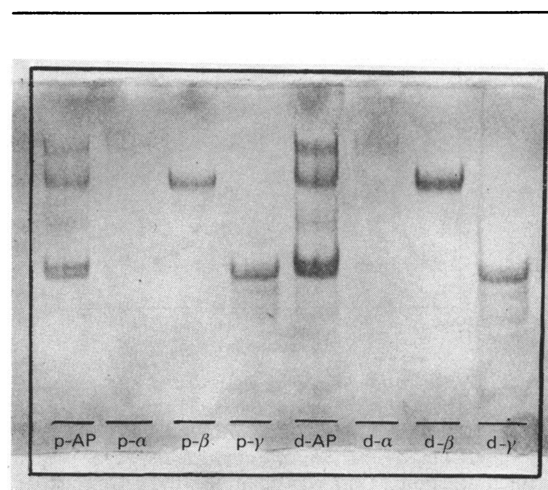


Fig. 1. *Gel electrophoresis in the presence of 1% SDS of reduced and carboxymethylated p- and d-aminopeptidases (p-AP and d-AP) and of both forms of the previously separated polypeptides (p-α, p-β and p-γ, and d-α, d-β and d-γ)*

The bands were revealed by protein staining. For experimental details see the text.

difficult, and these polypeptide chains were purified by Sepharose 4B filtration as indicated in Fig. 2.

Molecular weights of subunits and fragments

The use of the Fairbanks system for determination of molecular weights of the three polypeptides led to values (130000, 90000 and 45000) very similar to those obtained previously (Maroux *et al.*, 1973) by the technique of Weber & Osborn (1969). In both cases, the reference proteins were those mentioned in Fig. 2, reduced and carboxymethylated bovine trypsinogen (mol.wt. 24500) and pig pepsin (mol.wt. 34000) being added to improve the calibration of the lower-molecular-weight region. The same molecular-weight values were obtained for α- and β-polypeptides (130000 and 90000) when determined at acrylamide concentrations in the gels ranging from 5% to 9% and with the use of Ferguson (1964) plots. However, in these experiments, a higher value (65000) was calculated for the γ-polypeptide. This latter agrees well with that derived from Sepharose 4B filtration. Therefore the true molecular weight of the γ-polypeptide is still uncertain. As shown by Fig. 1, the p-γ- and d-γ-polypeptides yielded by gel electrophoresis two adjacent bands, suggesting the presence of at least two other polypeptides. Since a single *N*-terminal residue was always found in the preparations, this slight heterogeneity probably results from uncontrolled degradations in the *C*-terminal region of the γ-polypeptide. A similar heterogeneity was also detected by crossed immunoelectrophoresis (Fig. 3). In all electrophoretic systems used, the d- and p-forms of each of the three polypeptides had the same mobility.

Origin of the β- and γ-polypeptides

That the β- and γ-polypeptides resulted from a limited degradation of the α-chain was proved by the two following observations. (*a*) The amount of β- and γ-polypeptides was highly variable from preparation to preparation, and this amount was inversely proportional to that of the remaining α-chain. Some of our preparations were even completely devoid of α-chain, indicating that both copies of this subunit type existing in the native dimer are exposed to limited proteolysis. (*b*) The three polypeptides resulting from reduction and carboxymethylation of d-aminopeptidase were submitted to crossed immunoelectrophoresis (see the Experimental

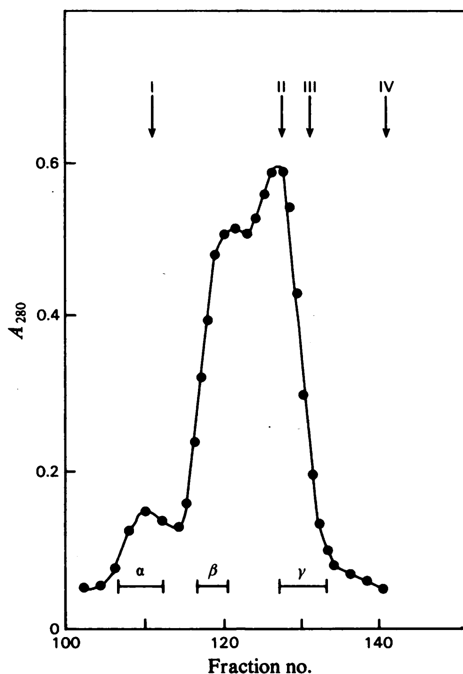


Fig. 2. *Gel filtration of 12mg of reduced and carboxymethylated p-aminopeptidase through a Sepharose 4B column (1.5cm × 200cm) equilibrated with 0.1 M-Tris/HCl buffer, pH 7.8, containing 0.1% SDS*

Each fraction (2ml) was analysed by gel electrophoresis. Fractions containing only one type of peptide were pooled as indicated by horizontal bars. Arrows indicate the elution volume of several reference proteins (I, rabbit p-aminopeptidase N; II, bovine serum albumin; III, catalase; IV, ovalbumin; respective molecular weights: 120000, 68000, 60000 and 43000). For experimental details see the text.

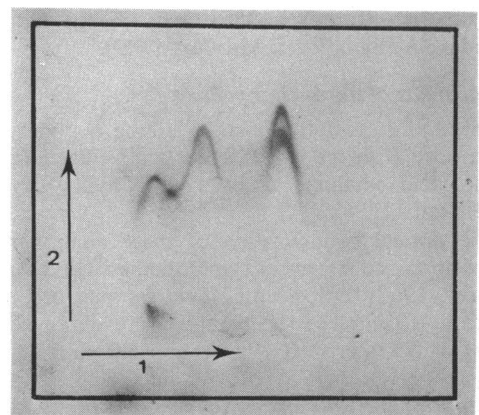


Fig. 3. *Crossed immunoelectrophoresis of reduced and carboxymethylated ^{125}I-labelled d-aminopeptidase N against guinea-pig anti-(p-α-chain)*

The migration of the three polypeptides in polyacrylamide gel is indicated by arrow 1. Arrow 2 indicates the migration in gel containing antibodies. The pattern was revealed by radioautography. For experimental details see the text.

section), with guinea-pig antibodies raised against purified α-chain. As shown by Fig. 3, three precipitation arcs were obtained, thus proving that β- and γ-polypeptides strongly cross-reacted with the anti-(α-chain) antibody. Crossed-immunoelectrophoresis patterns identical with that presented in Fig. 3 were also obtained with antibodies raised against the reduced and carboxymethylated complex (results not shown).

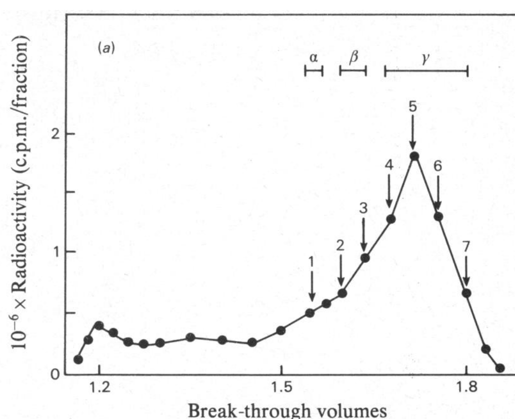
Presence of the anchor peptide in the d-α-chain and d-β-polypeptide

The presence of hydrophobic anchor peptides in the d-forms is known to confer on these forms amphiphilic properties that are lost in the corresponding p-forms. This difference allows an easy separation of the two forms by using a hydrophobic matrix such as phenyl-Sepharose or octyl-Sepharose (see the Experimental section). In the present work, the same difference in binding on phenyl-Sepharose was observed between α- and β-polypeptides from d- and p-forms dissociated by lithium di-iodosalicylate. This dissociating agent was chosen because of its weak denaturing capacities (Marchesi & Andrews, 1971; Choules *et al.*, 1973). Fig. 4 showed that it induced complete dissociation of the molecule.

Fig. 5 shows that the d-α- and d-β-polypeptides were strongly bound by phenyl-Sepharose whereas their p-counterparts were not retained. Considering that the yield from these columns was 90%, this result demonstrated that d-aminopeptidase did not contain α- and β-polypeptides without their hydrophobic anchors. Indeed, such subunits, being the same as the p-form, would not be retained on the phenyl-Sepharose column. Consequently, we conclude that the aminopeptidase molecule has two identical anchor peptides. Surprisingly, however, the d- and p-forms of the γ-fragment were also bound on the hydrophobic matrix. The origin of this abnormal behaviour may be assumed to be a higher sensitivity to denaturation by lithium di-iodosalicylate and/or to the existence of hydrophobic patches at the surface of the native chain. It is noteworthy that the behaviour of the γ-polypeptide during gel electrophoresis in the presence of SDS is also abnormal (see above).

N-Terminal residues in subunits and fragments

Alanine was the *N*-terminal residue in both d-α- and d-β-polypeptides, whereas valine was the *N*-terminal residue in p-α- and p-β-polypeptides. By contrast, d-γ- and p-γ-polypeptides has the same *N*-terminal residue, serine. In a previous study (Maroux & Louvard, 1976), alanine was also characterized as the *N*-terminal residue of the anchor peptide. These results strongly suggest that, as illustrated in Fig. 6, the pig intestinal aminopeptidase N molecule is anchored to the membrane by two hydrophobic peptides, corresponding to the *N*-terminal sequence of the d-α-subunit and d-β-polypeptide. The hydrophobic peptide corresponds to the *N*-terminal region of the undegraded α-chain.



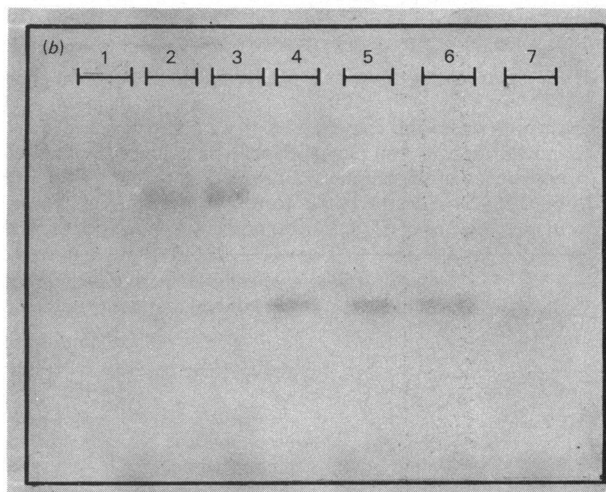


Fig. 4. *Filtration through Ultrogel AcA-34 of ^{125}I-labelled d-aminopeptidase after dissociation with di-iodosalicylate*

In (*a*), the material (2.5mg) was dissolved into 1ml of 0.1M-Tris/HCl buffer, pH 7.8, containing 0.1% Emulphogen and 50mM-lithium di-iodosalicylate. After a 5min incubation at 25°C, the solution was passed through a Ultrogel AcA-34 column (1cm × 200cm) equilibrated with the same buffer, except that lithium di-iodosalicylate concentration was 25mM. In (*b*), the analysis of 0.5ml fractions shown by numbered arrows in (*a*) by gel electrophoresis in SDS followed by radioautography. The fractions containing only one subunit type are indicated by horizontal bars. For experimental details see the text.

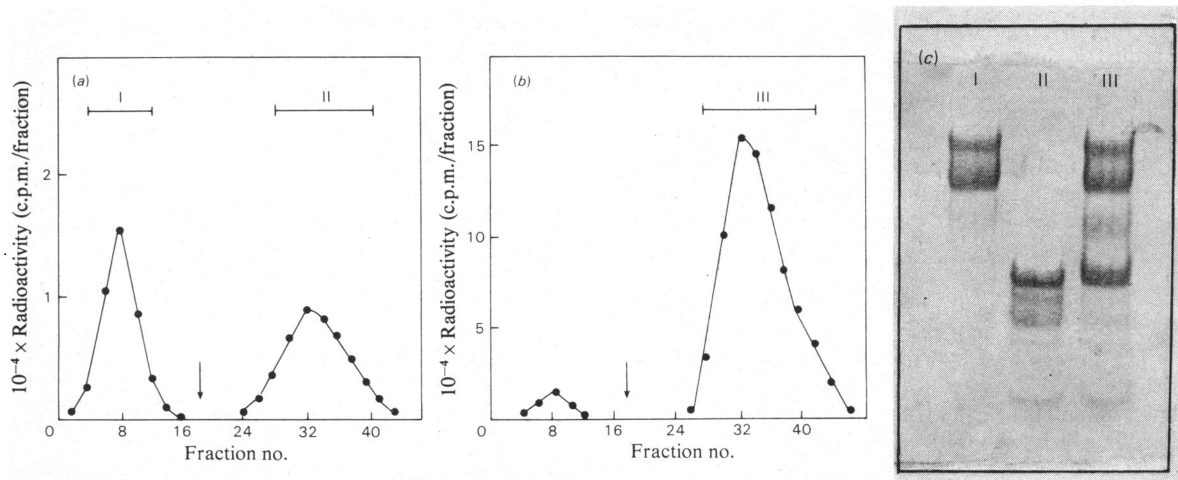


Fig. 5. *Chromatography on a phenyl-Sepharose column of the p-aminopeptidase (a) and of the d-aminopeptidase (b) after dissociation by di-iodosalicylate*

In both cases the enzyme (1 mg) was dissolved in 0.5 ml of dissociating buffer (see the text) and the solution was loaded on to a 1 ml phenyl-Sepharose column equilibrated and eluted with 0.1 M-Tris/HCl buffer, pH 7.8. After the emergence of the unretarded peak (peak I), the material remaining in the column (peaks II and III) was washed out by 0.2% SDS in the buffer (arrow). In (*c*) the three peaks were analysed by electrophoresis in polyacrylamide gel in the presence of SDS. For experimental details see the text.

Table 1. *Amino acid compositions of anchor peptides from several brush-border aminopeptidases*
The values are numbers of residues per peptide molecule, assuming a molecular weight of about 4000.

	Composition (residues/peptide molecule)		
	Pig		Rabbit
Amino acid	Aminopeptidase N anchor peptide (present work)	Aminopeptidase A anchor peptide (Benajiba & Maroux, 1980)	Aminopeptidase N anchor peptide (Feracci & Maroux, 1980)
Asp	3	4	3
Thr	2	2	1–2
Ser	3	3	3
Glu	3	5	4
Pro	1–2	2	1
Gly	4–5	5	6
Ala	3–4	3	3–4
Val	3	3	3
Met	0	1	0
Ile	2	2	2
Leu	3	4	4
Tyr	1	1	1
Phe	1	2	1
His	1	1	1
Lys	2	2	2
Arg	1	2	1
No. of residues	33–36	42	36–38
Mol.wt.	3450–3680	4530	3710–3880

The γ-polypeptide includes the *C*-terminal part of this chain.

Molecular weight, amino acid composition and optical configuration of the anchor peptide

Gel electrophoresis of the anchor peptide in the presence of SDS led to an apparent molecular weight of 8500 (Maroux *et al.*, 1973). As previously pointed out in similar cases (Feracci & Maroux, 1980; Benajiba & Maroux, 1980), this value may be overestimated, owing to an unusual accumulation of detergent around the hydrophobic site(s) of the

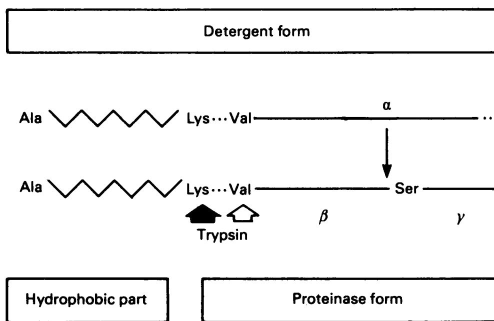


Fig. 6. *Tentative scheme for the conversion of the d-form of pig aminopeptidase N into the p-form and for the conversion of the α-subunit into β- and γ-fragments*
See Maroux & Louvard (1976) for an explanation of the trypsin hydrolysis.

peptide. Application of the isotope-dilution technique described previously (Benajiba & Maroux, 1980) yielded a value of 3500, assuming that two anchorage peptides were liberated per molecule of d-form converted into the p-form. This value compares well with those previously found for pig aminopeptidase A (4500; Benajiba & Maroux, 1980) and rabbit aminopeptidase N (3800; Feracci & Maroux, 1980). The amino acid compositions of the three aminopeptidase anchor peptides characterized so far are given in Table 1.

Alternation of D- and L-amino acids in polypeptides has been reported to generate pore-forming helical structures (Lotz *et al.*, 1976). This possibility was tested for the amino acids of the hydrophobic peptide, because of the hypothetical role that has been attributed to aminopeptidase in amino acid transport (Maroux & Louvard, 1976). However, all amino acids in the peptide were shown to be in the L-configuration.

Discussion

All brush-border hydrolases characterized so far as symmetrical dimers [pig intestinal aminopeptidase A (Benajiba & Maroux, 1980) and alkaline phosphatase (Colbeau & Maroux, 1978); pig renal dipeptidyl peptidase IV (Macnair & Kenny, 1979)] have been shown to be bound to the membrane by two anchorage peptides, whereas the hydrolases with different subunits [rabbit intestinal sucrose/isomaltase (Frank *et al.*, 1978) and rat kidney γ-glutamyltransferase (Hughey & Curthoys, 1976)] contain a single anchor peptide. This finding is of importance for a better understanding of the biosynthesis and mode of insertion of the brush-border enzymes. Asymmetrical dimers may be regarded as probably being derived from a high-molecular-weight precursor (Hauri *et al.*, 1979) synthesized by a single gene and split later into two parts by limited proteolysis.

Pig intestinal aminopeptidase, containing three polypeptides, represents a special case. The present work clarifies the position with regard to this subunit structure (Maroux & Louvard, 1976; Sjöström *et al.*, 1978; Norén & Sjöström, 1980). The three polypeptides were fully purified by gel electrophoresis and Sepharose 4B filtration in the presence of SDS. The β- and γ-polypeptides were shown to cross-react strongly with an antibody raised against the isolated α-chain, thus confirming the relationship existing between the three polypeptides. In our assays, the amounts of β- and γ-polypeptides were always much higher than those of the remaining α-chain, indicating that the two α-subunits composing the dimer were degraded by proteolytic attack. Consequently the aminopeptidase preparations probably contain a mixture of α_2, $\alpha\beta\gamma$ and $\beta_2\gamma_2$ complexes. Comparison of the *N*-terminal residues also showed that the β-polypeptide includes the *N*-terminal sequence of intact α-chain, and that the γ-fragment is from the *C*-terminal sequence.

Moreover, the existence of two anchor peptides per molecule of the d-enzyme form was proved in the present work by the following findings. (*a*) Both d-α-subunit and d-β-polypeptide contained an anchor peptide. They were bound by hydrophobic phenyl-Sepharose, whereas the corresponding p-forms were not retained. (*b*) α- and β-Polypeptides from the d-form and the anchor peptide have the same *N*-terminal residue, alanine, whereas valine is the *N*-terminal residue of α- and β-polypeptides from the p-form. (*c*) As with the pig intestinal aminopeptidase A investigated in this laboratory (Benajiba & Maroux, 1980), a molecular-weight value for the anchor peptide consistent with that found for the monomeric rabbit enzyme (Feracci & Maroux, 1980) could be obtained only when two molecules of anchor peptide per molecule of the d-enzyme form were assumed to be liberated during conversion into the p-form. As shown by Table 1, the molecular weights of the anchor peptides identified so far are between 3500 and 4500, suggesting that the total number of residues in these peptides does not exceed 33–42. The amino acid compositions of the three peptides are similar, suggesting a high degree of homology in this region of the molecules.

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References

Benajiba, A. & Maroux, S. (1980) *Eur. J. Biochem.* **107**, 381–388

Choules, G. L., Sanberg, R. G., Steggall, M. & Eyring, E. M. (1973) *Biochemistry* **12**, 4544–4550

Colbeau, A. & Maroux, S. (1978) *Biochim. Biophys. Acta* **511**, 39–51

Crestfield, A. M., Moore, S. & Stein, W. H. (1963) *J. Biol. Chem.* **238**, 622–627

Fairbanks, G., Steck, T. L. & Wallach, D. F. H. (1971) *Biochemistry* **10**, 2606–2617

Feracci, H. & Maroux, S. (1980) *Biochim. Biophys. Acta* **599**, 448–463

Ferguson, K. A. (1964) *Metab. Clin. Exp.* **13**, 985–1002

Frank, G., Brunner, J., Hauser, H., Wacker, H., Semenza, G. & Zuber, H. (1978) *FEBS Lett.* **96**, 183–188

Gorvel, J. P., Benajiba, A. & Maroux, S. (1980) *Biochim. Biophys. Acta* **615**, 271–274

Hauri, H. P., Quaroni, A. & Isselbacher, K. J. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5183–5186

Hughey, R. P. & Curthoys, N. P. (1976) *J. Biol. Chem.* **251**, 7863–7870

Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685

Lotz, B., Colonna-Cesari, F., Heitz, F. & Spach, G. (1976) *J. Mol. Biol.* **106**, 915–942

Louvard, D., Maroux, S., Vannier, Ch. & Desnuelle, P. (1975) *Biochim. Biophys. Acta* **375**, 236–248

Macnair, R. D. C. & Kenny, J. (1979) *Biochem. J.* **179**, 379–395

Marchesi, V. T. & Andrews, E. P. (1971) *Science* **174**, 1247–1248

Maroux, S. & Louvard, D. (1976) *Biochim. Biophys. Acta* **419**, 189–195

Maroux, S., Louvard, D. & Baratti, J. (1973) *Biochim. Biophys. Acta* **321**, 282–285

Meister, A. & Wellner, D. (1963) *Enzymes 2nd Ed.* **7**, 609–648

Norén, O. & Sjöström, H. (1980) *Eur. J. Biochem.* **104**, 25–31

Pagès, J. M., Louvard, D. & Lazdunski, C. (1975) *FEBS Lett.* **59**, 32–35

Sjöström, H., Norén, O., Jeppesen, L., Staun, M., Svensson, B. & Christiansen, L. (1978) *Eur. J. Biochem.* **88**, 503–511

Svensson, B. (1979) *Carlsberg Res. Commun.* **44**, 417–430

Weber, K. & Osborn, M. (1969) *J. Biol. Chem.* **244**, 4406–4412