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STRUCTURAL AND TOPOLOGICAL HOMOLOGY BETWEEN PORCINE INTESTINAL AND RENAL BRUSH BORDER AMINOPEPTIDASE

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SUMMARY

A method for the preparation of closed, right-side-out vesicles from the brush border membrane of the kidney proximal tubules is described. The aminopeptidase known to be bound to this membrane was investigated in order to compare its properties with those already reported for the intestinal enzyme. Both are composed of a hydrophilic, catalytically active part lying on the external side of the membrane and a short hydrophobic domain probably located in the N-terminal region of one of the subunits ensuring fixation to the lipid matrix.

The enzymes were also found to be chemically similar. Moreover, a quantitative immunological technique showed that they contained 6 cross-reacting determinants, consistent with a very high degree of homology. Four of these determinants were accessible in the bound form of the enzymes in the region of the active site. The other two, probably related to the junction between the hydrophilic moiety and the hydrophobic anchor were completely masked in the bound form. The remainder (6 in the intestinal and 4 in the renal enzyme), were heterologous.

The accessibility of two well defined determinants in this latter group was substantially reduced, perhaps by the proximity of the lipid and/or of other enzyme molecules.

The major function of the cells of the kidney proximal tubules and of the enterocytes is to absorb sugars, amino acids and salts. Both types of cell are equipped with a well developed brush border adapted to their common function.

The intestinal brush border membrane has been reported for many years to contain several hydrolase activities probably involved in the last steps of intraluminar digestion. It is noteworthy that most of these activities (aminopeptidase, alkaline phosphatase, maltase, trehalase and γ-glutamyltranspeptidase) are also present in the renal membrane where their role is not yet fully understood.

The intestinal [1] and renal [2] aminopeptidases have been purified to homogeneity. They are known to have about the same molecular weight, to be rich in sugars, to contain 2 Zn atoms per mol and to yield electrophoretic patterns in sodium dodecyl sulfate consistent with the presence of 3 different subunits in the molecules. These

findings suggest that the two proteins may be homologous. More recently, the intestinal aminopeptidase was shown [3] to be an amphipathic molecule extractable from the membrane by neutral detergents (the detergent form*). Upon digestion with papain or trypsin, this form yields a large, enzymatically active and hydrophilic part (papain or trypsin form*) and a short, strongly hydrophobic peptide of about 80 residues [4]. The first was shown by immunological techniques to protrude in situ almost entirely from the external surface of the membrane [5] whereas reconstitution assays [6] indicated that the hydrophobic domain corresponding to the peptide was probably plunged into the lipid bilayer and served as an anchor. In other words, the complete intestinal aminopeptidase appears to be a strictly oriented molecule, the hydrophilic part pointing towards the lumen and the hydrophobic part penetrating the lipid bilayer of the membrane.

The purpose of the present work is to confirm by chemical and immunological techniques that the intestinal and renal aminopeptidases are homologous molecules and to show that their position in the membrane as well as their mode of fixation are the same.

MATERIALS AND METHODS

Number of exposed antigenic determinants on a protein antigen. An immunological technique has recently been worked out in this Laboratory for quantitatively evaluating the portion of the surface of any membrane proteins protruding from the lipid bilayer [5]. It includes the preparation of an antibody monospecific for the t-aminopeptidase and the separate determination of the number of determinants that can simultaneously be saturated by the antibody in the trypsin-solubilized and bound form of the protein. This technique has now been improved [7, 8] by labelling the antibody with radioactive iodine [9] rather than with peroxidase. Moreover, the antigen concentration is now kept constant at about 10^{-6} M and increasing concentrations of antibody (usually 10^{-6}–10^{-5} M) are added in order to cover the whole range in which saturation is expected to take place. This range can be roughly anticipated knowing the molecular weight of the antigen [5]. All incubations are carried out for 2 h at 37 °C and then for 16 h at 4 °C in 10 μM dipotassium phosphate buffer 150 mM in NaCl and adjusted to pH 7.3 with phosphoric acid. The mixtures are centrifuged at 18 000 $\times g$ for 30 min and the unbound antibody is measured by counting radioactivity in the supernatants. The interpretation of the results according to whether the titrated antigen is, or is not the immunogen will be discussed later.

Depletion of monospecific antibody preparations raised against the t-aminopeptidase by the membrane-bound enzyme. Anti-intestinal and renal t-aminopeptidase antibody preparations were incubated for 2 h at room temperature and then for 16 h at 4 °C with a large excess of their respective membrane-bound antigen (antibody: antigen molar ratio about 0.8). After a 1-h centrifugation at 105 000 $\times g$, the supernatants could be expected to contain exclusively the antibodies specific for the masked determinants in the bound form and, consequently, to permit their specific titration in the trypsin form (see later).

* The aminopeptidase solubilized by detergent, trypsin or papain is designated d-aminopeptidase, t-aminopeptidase or p-aminopeptidase, respectively.

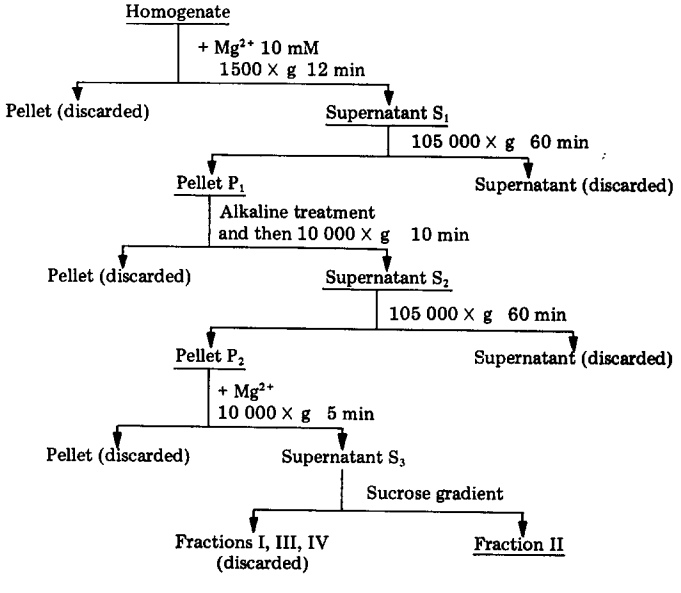
Isolation of heterologous antibodies in an anti renal t-aminopeptidase antibody preparation. The antibody preparation raised against the renal t-aminopeptidase was passed through a column containing the immobilized intestinal enzyme [5]. This column could be expected to retain specifically the antibodies directed against homologous regions in both molecules (57 % of the total antibody preparation). The eluate was used for titrating the heterologous determinants of renal aminopeptidase.

Enzyme activities. The techniques used for the determination of aminopeptidase, alkaline phosphatase and cytochrome reductase have already been described in previous publications [1, 3, 10]. Lysosomal acid phosphatase was measured as indicated by Torriani [11].

Preparation of closed vesicles from porcine kidney brush border. The technique currently used in this laboratory for the intestinal brush border [10] and that described by Booth and Kenny [12] for the rabbit renal brush border were combined. The main steps listed in Table I were monitored, as indicated in Table II, by the determination of five enzyme markers assumed to be characteristic of the brush border (aminopeptidase and alkaline phosphatase) and other subcellular organelles such as mitochondria (cytochrome oxidase), endoplasmic reticulum (NADPH cytochrome reductase) and lysosomes (acid phosphatase.)

TABLE I

FLOW SHEET OF THE PURIFICATION OF MEMBRANE VESICLES FROM RENAL CORTEX



The renal cortex, fresh or stored frozen at −80 °C, was sliced and homogeneized for 30 s in 6 times its weight of a hypertonic buffer (2 mM Tris · HCl at pH 7.1 containing 10 mM mannitol) with the aid of a Servall Omnimixer set at half its maximal speed. After filtration through gauze, the homogenate was adjusted to 10 mM in $MgCl_2$ and stirred for 15 min at 4 °C to aggregate the rough endoplasmic

TABLE II

COMPOSITION OF FRACTIONS DURING THE PURIFICATION OF THE RENAL BRUSH BORDER

See Table I for definition of fraction. For each enzyme, the first figure indicates the number of units per 100 units in the initial homogenate. The second figure in parentheses gives the purification factor of the enzyme in the fractions. All figures are the average of 3 independent assays. *A*, absorbance. Proteins are measured according to the method of Lowry et al.

Fraction	Proteins	Amino-peptidase	Alkaline phosphatase	Acid phosphatase	Cytochrome oxidase	Cytochrome reductase	A_{280}/A_{260}
Homogenate	100	100 (1)	100 (1)	100 (1)	100 (1)	100 (1)	0.82
Supernatant S_1	33.5	59 (1.74)	57.5 (1.7)	73.3 (2.2)	3.2 (0.1)	30 (0.9)	0.87
Pellet P_1	14	49.5 (3.54)	49 (3.5)	38 (2.7)	3.2 (0.2)	27 (2)	1
Supernatant S_2	2.8	28.9 (10.11)	27 (9.5)	33 (12)	0.5 (0.2)	5.5 (2)	0.59
Pellet P_2	2.8	28.7 (10.2)	24 (8.6)	2.7 (1)	0.4 (0.15)	4.9 (1.7)	1.1
Supernatant S_1	2.3	26.8 (12)	21 (9)	2 (0.9)	0	2.5 (1)	1.09
Fraction II	0.6	9 (15)	7.9 (13)	0.9 (1.4)	0	0.54 (0.9)	1.29

reticulum [13]. In this hypertonic medium, a single centrifugation at $1500 \times g$ for 12 min yielding supernatant S_1 was as efficient as the 2 centrifugations at $3000 \times g$ and $10\,000 \times g$ formerly used at this stage for the intestinal material [10]. Then, the mixture was centrifuged at $105\,000 \times g$ for 60 min to yield pellet P_1. The alkaline treatment of P_1 [10] was performed in the presence of 2 mM $CaCl_2$. This treatment was followed by a $10\,000 \times g$ centrifugation for 10 min to remove the aggregated endoplasmic reticulum. The $10\,000 \times g$ supernatant S_2 obtained was centrifuged at $105\,000 \times g$ for 60 min to yield pellet P_2. P_2 was resuspended in a 0.17 M NaCl, 10 mM Tris · HCl buffer pH 7.8, adjusted to 10 mM in $MgCl_2$, stirred for 5 min at 4 °C and centrifuged at $10\,000 \times g$. The supernatant S_3 obtained was further fractionated on a sucrose gradient.

For this purpose, all sucrose solutions were made 10 mM in a Tris · HCl buffer at pH 7.8, 5 mM in $MgCl_2$ and 20 mM in CsCl. Supernatant S_3 was adjusted to 30 % sucrose (w/w) by drop-by-drop addition of 60 % sucrose solution. Then the same step gradient as for the intestine was set up in the tubes of a Spinco SW 27 rotor: 6 ml (55 %), 10 ml (35 %), 10 ml (30 %) in which the membranes were suspended, 6 ml (25 %), 3 ml (10 %), the figures in parenthesis indicating the weight concentration of sucrose. The bulk of the suspended material was separated by a 5-h centrifugation at 22 000 rev./min at 4 °C into 4 main bands. The fraction remaining in the initial 30 % sucrose, marked Fraction II, contained the highly purified brush border membrane. The overall yield of the preparation was 8–9 % with a practically complete removal of contaminating endoplasmic reticulum and lysosomes.

Electron micrographs of the material in Fraction II are reproduced in Fig. 1. As in the case of the intestine [10], this material is essentially composed of closed vesicles. But their diameter is less uniform (0.1–0.4 μ).

RESULTS

Solubilization of renal aminopeptidase and other renal brush border hydrolases by neutral detergents and papain

The porcine renal brush border is devoid of maltase and sucrase activity. But, like the intestine of the same species, it contains an aminopeptidase, an alkaline phosphatase, a γ-glutamyl transpeptidase and a trehalase. The data presented in Table III suggest that the levels of aminopeptidase and trehalase are similar in both types of membrane, whereas the γ-glutamyl transferase and alkaline phosphatase are more abundant in the kidney and the intestine, respectively. However, the significance of

TABLE III

SPECIFIC ACTIVITIES (nmol/min per mg PROTEIN) OF SOME HYDROLASES IN RENAL AND INTESTINAL BRUSH BORDER MEMBRANE

The results of 5 independent assays were averaged in each case.

	Amino-peptidase	Alkaline phosphatase	γ-glutamyl transpeptidase	Trehalase
Kidney	2000	307	580	520
Intestine	2100	620	330	510

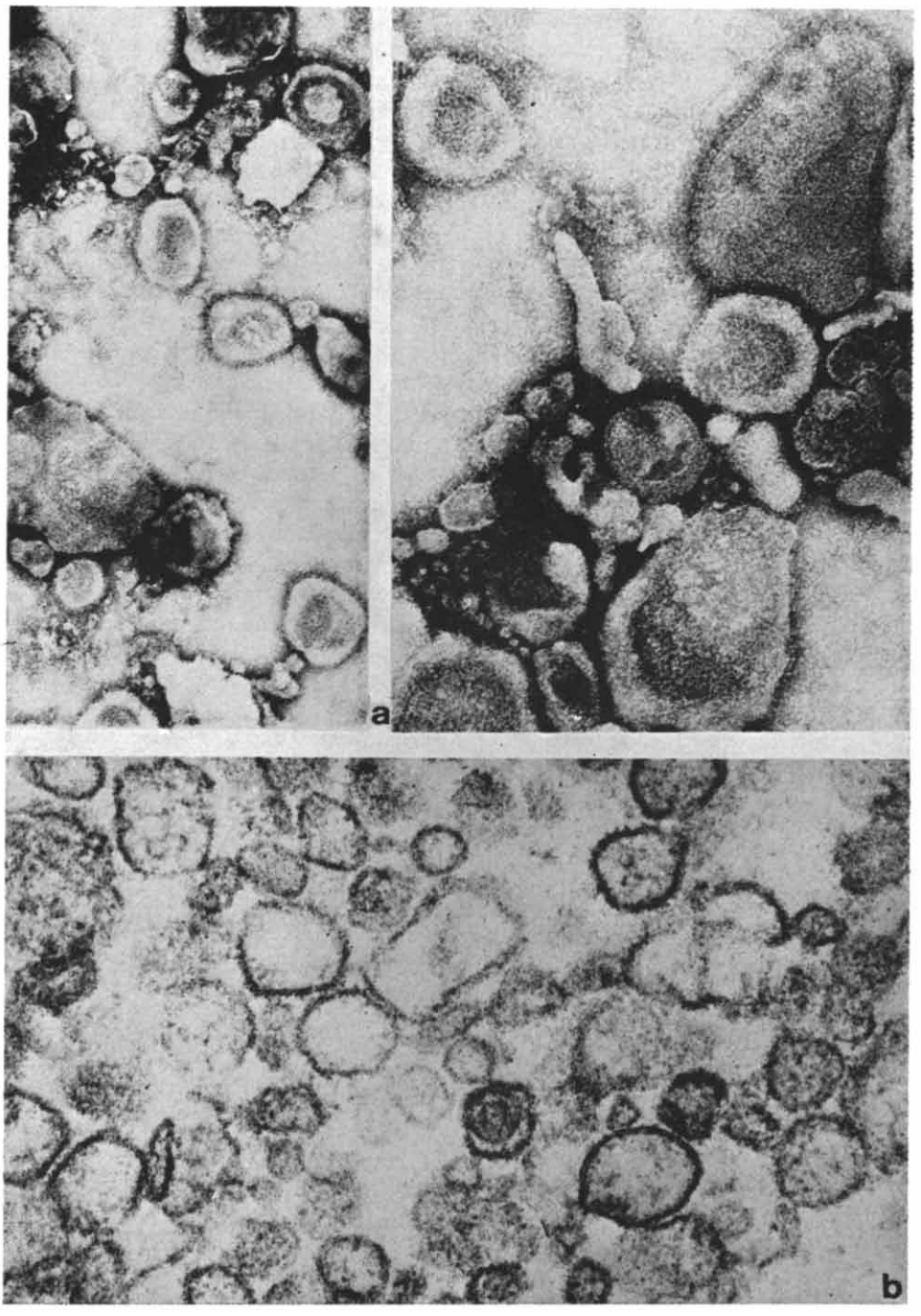


Fig. 1. Electron micrograph of Fraction II (Tables I and II). a: negative staining, magnification ×45600 (left) and ×91200 (right). b: thin section of the pellet containing fraction II, magnification ×91200.

these comparative results is lowered by the fact that the enzyme levels in the intestinal brush border may vary within large limits (3–4 fold) from lot to lot.

Then the ability of the renal enzymes to be solubilized by papain digestion of the vesicles or treatment with neutral detergents was compared to that of the intestinal material. It was quite remarkable in this respect that most of the renal enzymes were much more resistant to papain which released only 15 % of the total proteins of the vesicles instead of 50 % for the intestine [3]. This relatively poor effect of papain was confirmed by electron microscopy which showed that the granular aspect of the renal vesicles was largely conserved after digestion. Intestinal vesicles are known to take a smooth appearence under the same conditions [3].

Moreover, only aminopeptidase could be identified in the soluble fraction resulting from papain digestion of the renal vesicles. The release of the enzyme was complete and as fast as that of the intestinal aminopeptidase [3]. Thus, the behaviour of the renal aminopeptidase towards papain is completely atypical when compared to the other renal hydrolases for which no conditions permitting their solubilization by proteolysis have so far been found.

When renal vesicles were incubated overnight in a 2.5 % solution of the neutral detergent Emulphogen BC 720 at 4 °C, the aminopeptidase and γ-glutamyl transpeptidase activities were readily solubilized in their entirety whereas the yields for trehalase and alkaline phosphatase did not exceed 32 % and 3 % respectively. A point of great interest was that the detergent form of the renal aminopeptidase differed from the papain-released form by its strong tendency to aggregate and its R_f in gel electrophoresis. Furthermore, in accordance with previous observations made on material of intestinal origin [3], the detergent form of renal aminopeptidase could be converted by trypsin digestion to a form (the trypsin form) indistinguishable from that directly released from the membrane by papain. In conclusion, the renal aminopeptidase is, like its intestinal counterpart, an amphipathic molecule probably composed of a hydrophilic catalytic moiety and a hydrophobic anchor.

The data obtained in the course of these assays were also consistent with a high degree of chemical homology between the intestinal and renal enzymes. Their trypsin and detergent forms migrated at the same rate during gel electrophoresis. The purification procedures worked out in this laboratory [1–4] for the two forms of the intestinal enzyme could be applied without modification to the renal enzyme and the final products displayed the same specific activity.

Molecular properties of the renal aminopeptidase as compared with those of the intestinal enzyme

The impression that the trypsin form of the renal and intestinal aminopeptidase are homologous was reinforced by the data presented in Table IV. Although some of these data have already been published [1, 2, 14], they were again determined under exactly the same conditions for both enzymes in order to facilitate the comparison. The two trypsin forms have the same extinction and sedimentation coefficients. Their molecular weight and the composition of their sugar moieties are similar within experimental error. Both yield by gel electrophoresis in 1 % SDS 3 bands indicative of the existence of 3 subunits with molecular weights of 130 000, 96 000 and 49 000. Also only 3 bands are observed by charging the gel with a mixture of the two proteins. Furthermore, 3 N-terminal residues can be identified in both

enzymes, confirming the above-mentioned existence of 3 subunits. Two of these residues are the same in the trypsin and detergent forms, while the other (underlined in the Table IV), is different. A similar N-terminal residue substitution has been related in the case of the intestinal aminopeptidase to the cleavage of a hydrophobic peptide corresponding to the N-terminal region of one of the subunits of the molecule.

TABLE IV

COMPARISON OF SOME MOLECULAR PROPERTIES OF RENAL AND INTESTINAL AMINOPEPTIDASE

	Aminopeptidase	
	Renal	Intestinal
$E_{1\ cm}^{1\ \%}$ at 280 nm	15.9	15.6
$s_{20,w}$ (in Svedberg units)	9.98	9.95
Molecular weight* (Yphantis)	233 000	248 000
Sugars (%) in the trypsin form		
neutral	14	15
amino	7	8
sialic acids	1.5	0.3
No. of subunits		
(as suggested by electrophoresis of the trypsin form)	3	3
Molecular weight of subunits	130 000	130 000
	96 000	96 000
	49 000	49 000
N-terminal residues**		
in the detergent form	Gly	Ala
	Ser	Ser
	Gly	Ala
in the trypsin form	Gly	Ala
	Ser	Ser
	Val	Val

* Taking $\bar{\nu} = 0.693$ for both proteins.
** From ref. 4 for intestinal aminopeptidase.

Characterization of an hydrophobic domain in the detergent form of renal aminopeptidase

This peptide was purified to electrophoretic homogeneity by the same technique as that previously used for the material of intestinal origin [4]. It includes the labelling of the intact detergent form with radioactive iodine to facilitate the subsequent detection of the split products, trypsin digestion and two chromatographies of the resulting mixture on Sephadex G 50 and DEAE cellulose. The amino acid compositions given in Table V show that, although different, the renal and intestinal peptides have several common characteristics. Both are relatively short sequences (molecular weight about 9000) containing a high proportion of apolar residues (polarity indices 37 and 35) and devoid of disulfide bridges.

TABLE V

AMINO ACID COMPOSITION OF THE HYDROPHOBIC PEPTIDES FROM RENAL AND INTESTINAL AMINOPEPTIDASE

	Aminopeptidase	
	Renal	Intestinal*
Ala	11	11
Arg	3	1
Asx	6	3
½-Cys	0	0
Glx	6	5
Gly	9	9
His	1	1
Ile	9	9
Leu	10	10
Lys	4–5	5
Met	—	0–1
Phe	3	3
Pro	2	2
Ser	7	5
Thr	4	3
Tyr	2	4
Val	7	9
Total number of residues**	85	80
Total weight**	8970	8460
Polarity index**	37	35

* From ref. 4.
** Tryptophan not included.

Homology between the renal and intestinal aminopeptidases as estimated by immunological cross-reactions

The level of immunological homology existing between the trypsin form of the enzymes was estimated by titration of their common determinants with the aid of labelled, monospecific antibodies. As shown by Table VI, the anti-intestinal t-aminopeptidase antibody saturates, as reported earlier [5], a maximum of 12 determinants in its own antigen, whereas it combines with 6 determinants in the renal enzyme. With the anti-renal t-aminopeptidase antibody, the corresponding figures are 10 and 6. Hence, the two enzyme molecules possess 6 common determinants. Some examples of titration curves from which the above results were derived, are given in Fig. 2 A and B.

Study of the masked determinants in the bound form of the intestinal and renal aminopeptidases

Titrations with complete antibody. Table VI shows in the first place that the accessible surface of the membrane-bound intestinal aminopeptidase is saturated by a maximum of 8 antibodies instead of 10 as previously reported [5]. This discrepancy is due to the fact that, in all assays using the membrane-bound form of the enzyme, the antibody molecules remaining in solution below saturation were considered inactive whereas they actually corresponded to the masked determinants. This led to an unjustified correction and an overestimation of the titratable determinants in the

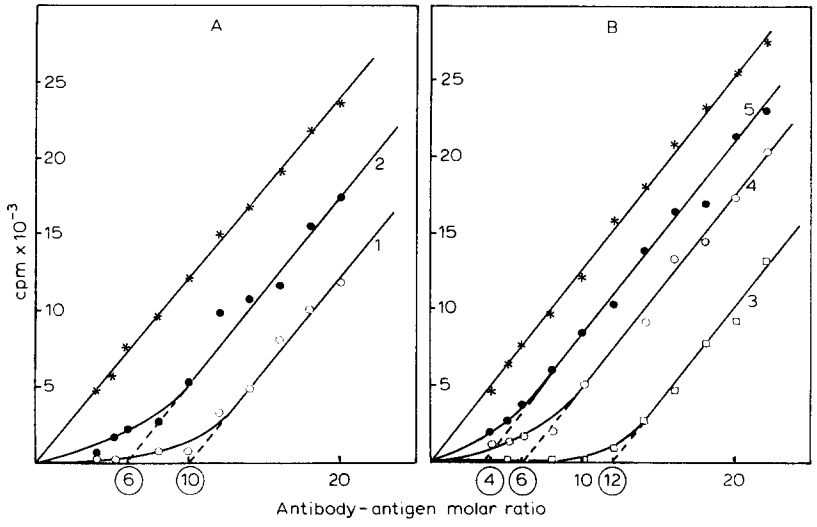


Fig. 2. Titration of antigenic sites in renal and intestinal aminopeptidases (trypsin form and membrane-bound) by complete monospecific antibodies. A: titrations with the anti-renal t-aminopeptidase antibody. Antigen: trypsin form or membrane-bound renal aminopeptidase (curves 1 and 2, respectively). The antigen (60 pmol) in a final volume of 100 μl of phosphate buffer was incubated with varying amounts of the labelled antibody (210–1320 pmol). The unbound antibody was determined by radioactivity counting in the 18000 $\times g$ supernatants. B: titrations with the anti-intestinal t-aminopeptidase antibody. Antigen: intestinal t-aminopeptidase (curve 3), renal t-aminopeptidase (curve 4), membrane-bound renal aminopeptidase (curve 5). Same experimental conditions as above. The stars indicate the antibody concentration in supernatant in the absence of antigen.

bound form. Table VI and Fig. 2A (curve 2) also indicate that 6 determinants are titrated under the same conditions in the bound renal aminopeptidase. In other words, integration in the membrane appears to reduce the number of titratable determinants from 12 to 8 for the intestinal aminopeptidase and from 10 to 6 for the renal aminopeptidase. The difference being 4 masked determinants in both cases, the two enzymes may be assumed to have about the same position on the external side of the membrane.

Titrations with depleted antibody. The antibody preparations raised against the t-aminopeptidases were depleted by a large excess of the bound antigen and used for the direct determination of the masked determinants in trypsin-solubilized forms. Table VI and Fig. 3 show that 2 masked determinants are titrated in this manner instead of 4 as found above by difference. These 2 determinants count among the 6 previously shown to be common to both enzymes.

The above-noted discrepancy between 4 and 2 masked determinants in the bound enzyme according to the technique used for their evaluation may be explained as follows: all the antibodies except those specific for 2 fully masked determinants are removed during the depletion of the antibody solution which is performed with a very large antigen excess. By contrast, the other 2, which react under the conditions of the depletion, remain quiescent during the titration where the antigen excess around saturation is much lower.

In order to know whether these latter determinants with an apparently poor

TABLE VI

NUMBER OF ANTIGENIC DETERMINANTS IN TRYPSIN FORM AND MEMBRANE-BOUND RENAL AND INTESTINAL AMINOPEPTIDASES

		Renal t-aminopeptidase antibody		Intestinal t-aminopeptidase antibody	
		Complete	Depleted	Complete	Depleted
Renal aminopeptidase	Trypsin form	10	2	6	2
	Bound	6	0	4	0
Intestinal aminopeptidase	Trypsin form	6	2	12	2
	Bound	—	—	8	0

accessibility occupy well defined positions on the enzyme surface, an anti-renal t-aminopeptidase antibody preparation was freed, as indicated under Materials and Methods, of all the molecules reacting with the intestinal t-aminopeptidase. In this manner, the number of titratable determinants in the renal t-aminopeptidase was, as expected, reduced from 10 to 4 (Fig. 4). But, the important point was that only 2 determinants were again titrated in the bound form, strongly suggesting a steric hindrance effect in a limited and well defined portion of the molecule. In other words, this portion is likely to contain 4 determinants and the saturation of 2 of them hampers the saturation of the other 2. A similar representation is probably also applicable to the intestinal aminopeptidase.

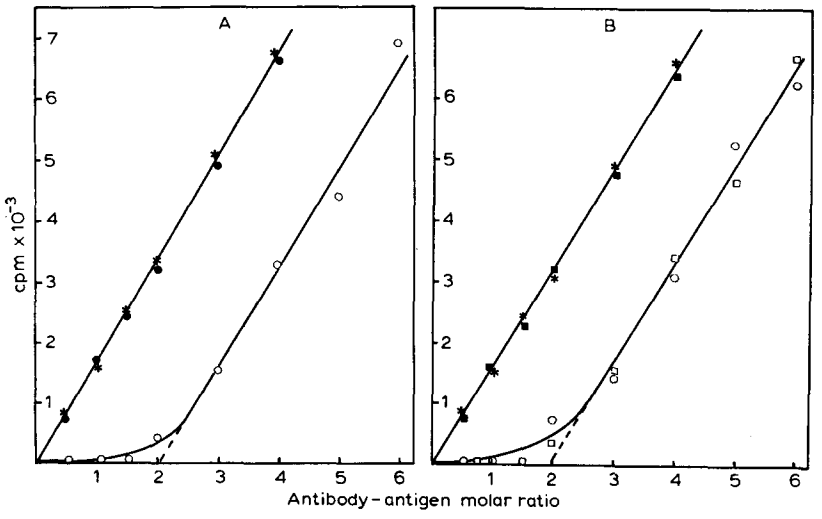


Fig. 3. Titration with antibodies depleted with the membrane-bound enzyme. A: anti-renal t-aminopeptidase antibody (5 pmol) depleted with the bound renal aminopeptidase and then incubated in 100 μl of phosphate buffer with 2.5–30 pmol of trypsin form (○) or bound (●) renal enzyme. B: same experiment with the anti-intestinal t-aminopeptidase antibody depleted with the bound intestinal aminopeptidase and incubated with the intestinal t-aminopeptidase (□), bound intestinal aminopeptidase (■) and renal t-aminopeptidase (○). The assays with the bound enzyme should obviously lead to no fixation. They served as blanks demonstrating the general consistency of the results.

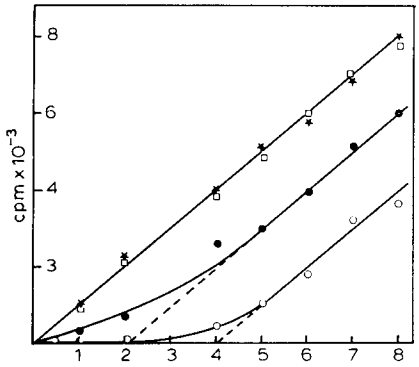


Fig. 4. Saturation of non-cross-reacting determinants in the trypsin form (○) and bound (●) renal aminopeptidase and intestinal t-aminopeptidase (□) by an anti-renal antibody depleted by the intestinal enzyme. The antigen (12 pmol) was incubated under the same conditions as before with varying amounts of depleted antibody (6–72 pmol). The stars indicate again the total amount of radioactivity in supernatant, in the absence of added antigen.

If the low reactivity of 2 determinants out of 4 in the renal (and probably also intestinal) enzyme is really due to steric hindrance, this effect may be assumed to result from the proximity of the membrane and/or from the close packing of enzyme molecules at the membrane surface [5]. It is probably significant that this effect persists when the bulky γ-globulins used as antibodies in all the preceding assays are replaced by Fab fragments.

Inhibition of aminopeptidase activity by anti-t-aminopeptidase antibodies

An antibody raised against the intestinal t-aminopeptidase was recently reported to inhibit its solubilized and bound antigen by 75 % and 50 %, respectively.

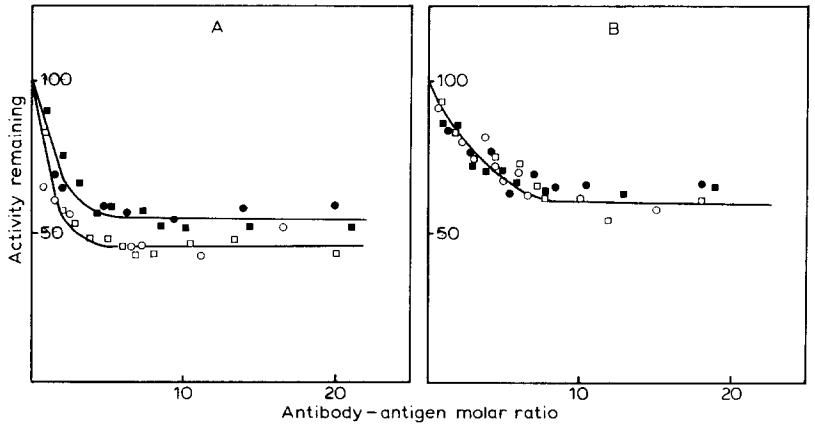


Fig. 5. Inhibitory effect of antibodies against intestinal (A) and renal (B) trypsin form (□) and bound (■) intestinal aminopeptidase, trypsin form (○) and bound (●) renal aminopeptidase. The enzyme (35 pmol) was incubated with varying amounts of antibody (30–700 pmol) in a final volume of 75 μl of phosphate buffer.

The present assays showed that this inhibition was in fact quite variable according to the antiserum tested. However, as shown in Fig. 5, they confirmed that the inhibition was never complete, the antibodies being probably bound in the vicinity of the active site rather than directly on it. Moreover, the results indicated that solubilized or bound enzymes were inhibited to about the same extent by their own antibody and that directed against the other enzyme. Therefore, the active site of aminopeptidase is probably located in a privileged region containing the 4 determinants both accessible in the bound form and common to the 2 enzymes.

DISCUSSION

The procedure proposed for the obtention of closed vesicles from the renal brush border membrane does not differ essentially from that recently worked out for the same material of intestinal origin [10]. However, probably because of a different net charge, the renal membrane was found to aggregate more easily in the presence of the divalent cations (Ca^{2+} or Mg^{2+}) used to facilitate the removal of the endoplasmic reticulum [13]. Special care must be taken at this stage to minimize the losses. The density of the renal vesicles (1.14) was also lower than that of the intestinal vesicles (1.20), the consequence being that the rough endoplasmic reticulum must be loaded with Cs^{+} ions [15] to permit their ready separation from the brush border membrane in the sucrose gradient. Another important difference at the level of the general organization of the membranes was the much higher resistance of the renal hydrolases towards solubilization by papain and neutral detergents. In fact, only the renal aminopeptidase could be solubilized by papain. This solubilization was complete showing that, like the intestinal vesicles, all the renal vesicles were right-side-out. The renal aminopeptidase was also much better solubilized by neutral detergents than the other enzymes bound to the same membrane. Another point of interest was that the detergent form of both renal and intestinal aminopeptidases were amphipatic molecules from which a short hydrophobic domain probably located in the N-terminal region of one of the subunits could be split off by papain or trypsin without impairing the catalytic activity of the remaining hydrophilic moiety. These domains liberated as a peptide were devoid of disulfide bridges and rich in apolar residues, these characteristics apparently facilitating interactions with a lipid environment [16]. The peptides from the renal and intestinal aminopeptidases had the same composition in apolar residues except for 2 valine and 2 tyrosine residues missing in the first. The conclusion at this stage is that the 2 aminopeptidases appear to be external surface constituents of their respective membranes and to be bound to the lipid matrix by a relatively short hydrophobic sequence in the N-terminal region of one of the subunits. The role of an anchor attributed to this sequence has been directly demonstrated in the case of the intestinal aminopeptidase by reconstitution assays [6].

Another point of interest was that a very high degree of homology between the renal and intestinal aminopeptidases could be demonstrated by chemical and immunological techniques. The detergent and trypsin forms of both enzymes had exactly the same behaviour during their respective purification. They were precipitated by the same ammonium sulfate concentration, they emerged at the same position from Indubiose and DEAE-cellulose columns and they displayed the same electrophoretic

mobility in polyacrylamide gels. Moreover, most of their molecular and catalytic properties were found to be identical or very similar.

Available chemical and crystallographic data show that most of the mutations due to protein evolution affect the residues at the surface of globular proteins while those present in the central hydrophobic core are largely invariant. As a consequence, immunological techniques, due to their known ability to explore the surface of the antigens [17] and also the strict specificity of the antibody-antigen interactions may be expected to be an extremely sensitive tool for estimating the evolutionary distance between homologous proteins. It has been reported that 2 homologous proteins must not differ by more than 30 % in their primary structure to cross-react immunologically [18].

The quantitative immunological technique worked out in this laboratory to study the position of intestinal aminopeptidase with respect to the lipid bilayer of the membrane [5] has been extended herein to an evaluation of the degree of homology existing between the trypsin forms of the intestinal and renal enzymes. By measuring the maximal number of antibody monospecific for one enzyme able to bind to the other, the 2 proteins were shown to possess as many as 6 common determinants out of a total of 12 and 10, respectively. Therefore, the homology between the 2 molecules is definitely demonstrated.

The other results obtained using complete and depleted antibody preparations are best illustrated by the tentative scheme reproduced in Fig. 6. Among the 6 determinants common to both enzymes, 4 are fully accessible and the corresponding region is likely to contain the active site of the catalytic moiety. The other 2, which are completely masked in the bound form of both enzymes but readily titratable in the trypsin form, are probably related to the region where the junction between the catalytic moiety and the hydrophobic anchor occurs. They may be masked by the lipid and become accessible after solubilization or be generated de novo on the surface of the trypsin form by the conformational changes resulting from the proteolytic splittings associated with solubilization. Finally, 4 determinants in the renal enzyme and 6 in the intestinal enzyme are heterologous. Two of them appear to have a reduced accessibility due perhaps to the proximity of the lipid surface and/or of other enzyme molecules.

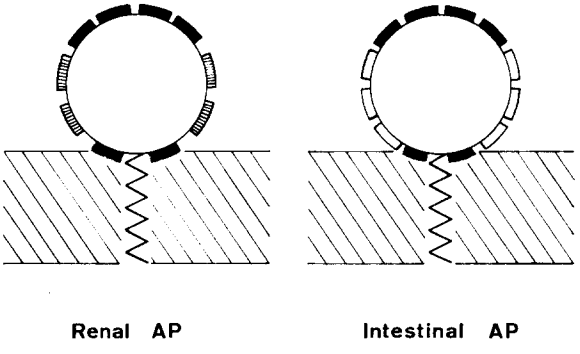


Fig. 6. Immunological homology between the renal and intestinal aminopeptidases (AP). The determinants are common and accessible (black), common and masked (grey) or heterologous (hatched or white).

The purpose of this representation, which is schematic and tentative, is to interpret in terms of molecular structure the observed fact that the antigenic determinants in both renal and intestinal aminopeptidases fall into 3 categories: common and fully accessible, common and fully masked and heterologous among which 2 have a reduced accessibility.

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