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SIMULTANEOUS ISOLATION OF BRUSH BORDER AND BASOLATERAL MEMBRANE FROM RABBIT ENTEROCYTES

PRESENCE OF BRUSH BORDER HYDROLASES IN THE BASOLATERAL MEMBRANE OF RABBIT ENTEROCYTES

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Summary

By a slight modification of the procedure described by Gratecos et al. (Gratecos, D., Knibiehler, M., Benoit, V. and Sémériva, M. (1978) Biochim. Biophys. Acta 512, 508—524), the basolateral and brush border membranes of rabbit enterocytes have been purified concomitantly from the same aliquot of mucosa. The two types of membrane have been obtained with the same yield (15%) and enrichment of specific markers (18-fold).

The presence in the basolateral membrane of hydrolases known to be specific of the brush border membrane has been confirmed by using immunological techniques.

Introduction

The plasma membrane of the enterocytes is composed of at least two regions characterized by their different morphological aspect and enzymatic equipment well adapted to their specific functions: the brush border membrane at the luminal pole of the cells and on the contra-luminal faces, the lateral and basal membranes.

During subcellular fractionation, the brush border membrane is easily differentiated from the contraluminal membranes and more generally from all the other membranes. Its luminal surface displays a characteristic granular appearance by electron microscopy and it contains a large quantity of digestive hydrolases (aminopeptidases, disaccharidases, alkaline phosphatase) considered as

specific markers [1,2]. In contrast, no marker strictly specific of the contraluminal membranes has yet been identified. However, these more classical plasma membranes contain two general enzymatic markers of plasma membranes: 5′-nucleotidase, which is present in very low amount in the brush border membrane, and, especially, the ($Na^+ + K^+$)-ATPase which is absent from this latter membrane [3,4].

It is important to emphasize here that the basal and lateral membranes are probably different, but no selective marker permits one to be distinguished from the other during subcellular fractionation. This explains the general use of the term 'basolateral' to designate both membranes taken together. In contrast to the basal membrane, the lateral membrane has been described as being folded into numerous plicae [5] and consequently the basolateral membrane preparations may contain essentially the lateral membrane.

The existence of specialized regions on the plasma membrane certainly plays an important role for the functional polarity of the enterocyte.

As will be discussed later, the localization of enzymes either restricted to one of these regions or rather widespread amongst all of them raises an important question in relation to the mechanism responsible for the formation of different domains in the plasma membrane during its biogenesis. In situ, no brush border hydrolase, in particular aminopeptidase N, has been detected in the basolateral membrane by the use of histochemical or immunochemical techniques [6]. In contrast, all the preparations of basolateral membranes contain these enzymes in small but not negligible amount [7–9]. Despite the good separation of the two types of membrane generally obtained, it is difficult to exclude some slight contamination of the basolateral membrane preparations by the brush border membrane that could explain these results.

By use of antibodies specific for rabbit aminopeptidase N and the brush border membrane itself [6], we have confirmed that the basolateral membrane contains small amounts of all the brush border digestive hydrolases.

Material and Methods

Analytical techniques. Membrane proteins were evaluated as previously described [9,10].

Enzyme activity determinations. Aminopeptidase activities were measured spectrophotometrically at 410 nm [11] with L-alanine *p*-nitroanilide as substrate (Bachem) in the case of aminopeptidase N and with L-α-glutamic acid-*p*-nitroanilide as substrate in the case of aminopeptidase A.

The techniques used for the determination of alkaline phosphatase, disaccharidases and cytochrome oxidase activities have been described in previous publications [10,12].

The assay conditions for the determination of 5′-nucleotidase activity have been devised to take into account the presence in the brush border of large amounts of alkaline phosphatase which can readily hydrolyze 5′-AMP. By working at pH 7.4 it is possible to determine accurately the 5′-nucleotidase activity in the presence of levamisole (Aldrich), an inhibitor of the alkaline phosphatase activity. The incubation medium consisted of 30 mM Tris-HCl (pH 7.4)/7.5 mM $MgCl_2$/1 mM $MnCl_2$/1 mM levamisole. After 5 min of preincuba-

tion, the reaction was initiated by the addition of 0.1 mM adenosine 5-monophosphate as substrate and was monitored at 265 nm in a coupled assay with excess adenosine desaminase (Boehringer) [14].

It is now well established that the ouabain sensitive ($Na^+ + K^+$)-ATPase also exhibits a K^+-dependent phosphatase activity which is still ouabain sensitive [15]. This activity was measured instead of the hydrolysis of ATP. The method described by Garrahan et al. [16] adapted by Murer et al. [8] was used with slight modifications. Two enzyme samples were placed in 0.5 ml of a buffer comprising 50 mM Tris-HCl (pH 7.6)/10 mM $MgSO_4$/5 mM EDTA/90 mM KCl and incubated for 30 min at 37°C either in the presence or in the absence of ouabain (0.7 mM). Each reaction was initiated by the addition of 0.5 ml of 12 mM *p*-nitrophenylphosphate (Sigma 104 phosphatase substrate) solution in the same buffer prewarmed 5 min at 37°C. After incubation for 30 min at 37°C the reaction was stopped by adding 0.2 ml of 30% trichloracetic acid and the samples were centrifuged when necessary. The amount of *p*-nitrophenol thus released was then measured at 410 nm after the addition of 2 vols. of 1 M Tris-HCl. The ouabain sensitive K^+-phosphatase activity was computed by subtracting the value found in the presence of ouabain from that found in the absence of ouabain.

Ouabain (Strophantin G octahydrate, Sigma) was dissolved in the Tris-HCl buffer on the day of use. An enzyme unit is the amount of enzyme catalyzing the hydrolysis of 1 nmol substrate per min.

Immunization. Guinea pigs were immunized as previously described [6] with 2.7 mg protein sample of Emulphogen (G.A.F. France) extract from basolateral membranes I and II and from brush border membrane (see Table I). The immunoglobulins were purified from the sera by $(NH_4)_2SO_4$ precipitation and DEAE-Sephadex chromatography [17].

Goat immunoglobulins from a serum raised against rabbit aminopeptidase N were a gift from H. Feracci. These anti-aminopeptidase antibodies were specific as judged by the crossed-immunoelectrophoresis technique. They reacted only with the brush border [6] when tested by the sandwich-immunofluorescence technique on ultrathin frozen sections of rabbit intestinal mucosa.

Crossed-immunoelectrophoresis. This technique as well as the subsequent revelation and identification of immunoprecipitates have been previously described in detail [18].

Electron microscopy. Samples for thin sections were prepared as described in Ref. 9. Negatively stained material was prepared as in Ref. 10 except that a 1% solution of phosphotungstic acid in water adjusted to pH 7.2 replaced uranyl acetate.

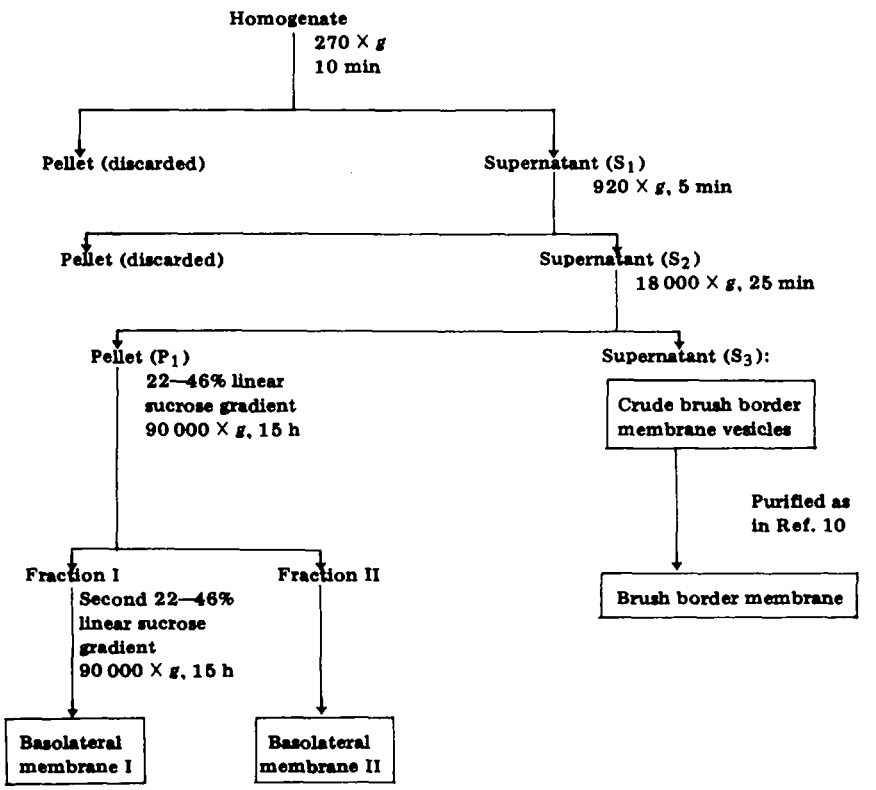
Centrifugation methods. A Sorvall superspeed refrigerated centrifuge model RC 2 B equipped with a SS 34 angle rotor (8 × 50 ml) was employed for low-speed centrifugations. Preparative high-speed centrifugations were performed in the 42 Ti angular rotor of a Spinco-Beckman centrifuge model L5-65. Gradient centrifugations were also performed in a Spinco Beckman centrifuge equipped with a swinging bucket rotor SW 27. All *g* forces were calculated at the bottom of the tubes.

Percoll gradients were formed as recommended by Pharmacia. The membrane sample was mixed with 8.4 ml of Percoll (Pharmacia) and brought to 35

ml with 10 mM phosphate (pH 7.4)/0.15 M NaCl buffer. The mixture was centrifuged at 15 000 rev./min for 20 min in the SS 34 angle rotor of a Sorvall centrifuge.

Preparation of plasma membranes. The technique described by Gratecos et al. [9] for rat intestinal mucosa was used with some modifications. In the case of rabbit membranes the main differences were in the centrifugation steps which permitted partial separation of the basolateral from the brush border membrane. This procedure led to the complete purification of these two types of membrane from the same starting aliquot of mucosa.

The mucosa was gently scrapped with a microscope slide and the collected scrapings (approx. 20 g from three rabbits) were suspended under mild magnetic stirring in volumes representing 8 times their weight of a buffer comprising 10 mM Tris-HCl (pH 7.4)/5 mM $MgCl_2$/0.15 M sucrose. The suspension was then supplemented with ions required for loading mitochondria: 14 ml 70 mM NaH_2PO_4 was added to obtain a final concentration of 5 mM and the pH was adjusted to 7.4 with NaOH. The succinate and $MnCl_2$ was brought to 30 mM and 1 mM, respectively, by the addition of 4 ml 1.4 M succinate buffer and 2 ml 100 mM $MnCl_2$.



Scheme I. Flow diagram for the purification of plasma membranes originating from rabbit intestinal mucosa.

TABLE I

COMPOSITION AND ENZYME CONTENT OF VARIOUS FRACTIONS FROM RABBIT INTESTINAL MUCOSA

The separation of the fractions is described in Scheme I. For each enzyme the first figure indicates the number of units found in the fraction per 100 units in the homogenate. The figure in parentheses gives the specific activity in units/mg protein. The figures are the average of eight assays, except for maltases and lactase for which a single determination was performed.

	5′-Nucleotidase	$(Na^+ + K^+)$-ATPase	Aminopeptidase N	Alkaline phosphatase	Maltases *	Lactase	Cytochrome oxidase	Proteins (mg)
Homogenate	100 (3.6)	100 (6.5)	100 (56)	100 (51)	100 (2 200)	100 (51)	100 (31)	100
Supernatant S_1	71 (4.4)	67 (7.5)	62 (60)	61 (52)	67 (2 500)	60 (54)	30 (16)	58
Supernatant S_2	69 (5.5)	61 (8.5)	58 (69)	50 (55)	52 (2 500)	48 (52)	9 (6)	47
Supernatant S_3	33 (3.2)	15 (2.6)	33 (50)	22 (31)	30 (1 800)	25 (35)	2 (1.6)	37
Pellet P_1	36 (13)	44 (29)	26 (145)	28 (143)	21 (4 600)	23 (100)	6 (18)	10
Fraction I	8 (65)	3 ** (42)	0.3 (37)	0.4 (45)	0.3 (2 000)	1.7 (130)	0	0.46
Fraction II	16 (67)	7 ** (54)	0.9 (6.1)	0.8 (48)	0.8 (2 700)	3 (190)	0	0.85
Brush border membrane	4 (20)	0	15 (1050)	14 (944)	15 (43 000)	11 (710)	0	0.80

* At least two enzymes giving immunoprecipitate 3 and 5 in Figs. 3 and 4 hydrolyze maltose.

** A loss of K^+-phosphatase, ouabain-sensitive activity is always observed over the sucrose gradient.

The suspension was homogenized by five strokes of a motor-driven Teflon-glass homogenizer (clearance 0.15—0.23 mm) operated at 1500 rev./min and filtered through gauze. The resulting homogenate was incubated for 30 min at 25°C with magnetic stirring to ensure loading of mitochondria. An additional homogenization was affected by two strokes of the pestle and the final homogenate was fractioned by several centrifugations as described in Scheme I.

The different types of membrane and subcellular organelle were characterized by the enzymatic markers used in Refs. 9 and 10. Only markers for mitochondria and basolateral and brush border membranes were systematically measured during the preparation.

As shown in Table I, the 18 000 × *g* centrifugation step resulted in a partition of the brush border enzymatic markers between the pellet, P_1, and the supernatant, S_3. The same partition was true for the basolateral markers. However, it was found that the subsequent purification of the basolateral membranes from P_1, and especially of the brush border membrane from S_3, led to preparations with higher specific activities and an equal yield than when both membranes were completely collected together in a 105 000 × *g* pellet from the supernatant S_2.

The brush border membrane vesicles were purified from the 18 000 × *g* supernatant S_3 by the technique routinely used in this laboratory [10], starting from the first 105 000 × *g* centrifugation step. As judged by the specific activities of the different markers (Tables I and II) the material obtained was identical to that prepared by the complete procedure described in Ref. 10.

The pellet P_1 collected after the 18 000 × *g* centrifugation was resuspended by hand homogenization in a minimum volume of a 22% sucrose solution containing 10 mM Tris-HCl (pH 7.4) and 2.5 mM $CaCl_2$ and layered on a 22—46% sucrose gradient as described in Ref. 9. As in the case of membranes from rat tissue [9,19], the basolateral membrane markers were partitioned into two major components: fraction I and fraction II localized at a sucrose concentration of 32—33% and 36—37%, respectively, well separated from the brush border membrane which was found at a 43% sucrose concentration.

These two fractions were submitted individually to a second sucrose gradient centrifugation and they behaved as apparent single species since they equilibrated at the same sucrose density as in the first centrifugation. They constituted the basolateral membrane I and II preparations.

TABLE II

SPECIFIC ACTIVITIES OF SOME HYDROLASES IN THE BASOLATERAL AND BRUSH BORDER MEMBRANES

Specific activities are expressed as nmol/min per mg protein.

Membrane	$(Na^+ + K^+)$-ATPase	5'-Nucleotidase	Alkaline phosphatase	Aminopeptidase N	Aminopeptidase A	Maltases *	Lactase
Basolateral I	64	89	50	56	81	2 400	150
Basolateral II	63	74	60	66	76	3 200	230
Brush border	0	20	944	1050	788	43 000	709

* At least two enzymes giving immunoprecipitates 3 and 5 in Figs. 3 and 4 hydrolyze maltose.

Results

(1) Structural features of the different membrane preparations

The basolateral and brush border membrane preparations were examined under the electron microscope after negative staining (Fig. 1) or on thin sections (Fig. 2). No significant difference could be observed between basolateral membranes I and II, and therefore micrographs of only one fraction are given.

Negative staining of the brush border membrane fraction revealed that the preparation was not exclusively composed of spherical vesicles as in Ref. 10 but intact microvilli also were present in high proportion.

As already observed [10] on thin sections of brush border membrane preparations, the vesicles or microvilli were partly filled with the fibrous material of the brush border. Some fibrous material was also visible in the basolateral membrane vesicles although in much lower amount. Its binding to the internal side of the membrane could not be clearly observed.

The most striking structural difference between the basolateral and brush border membranes was the aspect of their external surface: the basolateral membrane was smooth, whereas the brush border membrane was overlaid with an abundant fuzzy coat (see inserts, Fig. 2). This fuzzy coat could contribute to the very high density of the brush border membrane on sucrose gradient: 1.19 compared to 1.14 and 1.16 for the basolateral membranes I and II, respectively. In contrast, on high molecular weight Percoll gradients, the two fractions of basolateral membranes had the same density: 1.03. This value is very close to the density of the brush border membrane preparation of 1.04. The large difference between the densities observed by centrifugation in sucrose or Percoll gradients strongly suggest that the membrane preparations were composed of osmotically sensitive closed vesicles [20]. The same conclusion could be drawn from the electron microscopy observation.

(2) Precipitation of the brush border and basolateral membranes by specific anti-aminopeptidase N antibodies.

Samples of basolateral membranes I and II and of brush border membrane, each containing 50 units of aminopeptidase N were incubated for 2 h at 37°C in a total volume of 200 μl of 10 mM phosphate buffer (pH 7.4)/0.15 M NaCl with 50 μg of goat immunoglobulins. A 35% inhibition of the aminopeptidase activity present both in the brush border and in the basolateral membranes was observed. Then precipitation was induced by the addition of 195 μg of rabbit anti-goat γG-antibodies. After incubation (1 h at 37°C followed by 16 h at 40°C), the insolubilized material was separated by a 2 min centrifugation in an IEC bench centrifuge operated at full speed (about 600 × *g*). It was checked that native vesicles were not sedimented under these conditions. In addition, blank assays, in which the addition of anti aminopeptidase was omitted, were performed in parallel with each sample of membrane. The proportion of aminopeptidase N, 5′-nucleotidase and ($Na^+ + K^+$)-ATPase activities present in the sediment and in the supernatant was determined. As expected, no material was precipitated in the blank assays. The results are summarized in Table III.

As already found in the case of pig brush border membrane vesicles [21], 95% of the brush border membrane enzyme activities were precipitated. By

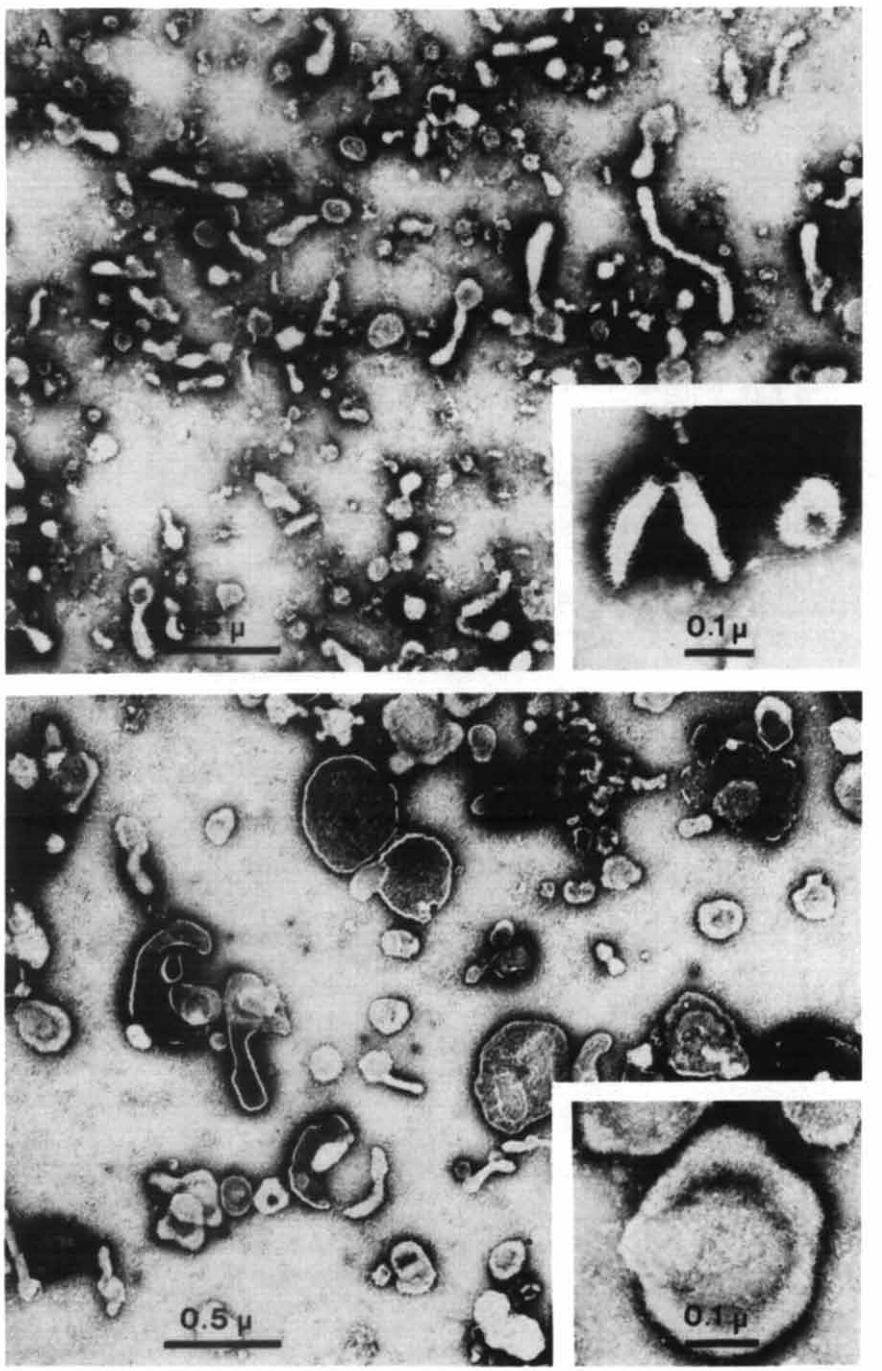


Fig. 1. Negative staining of brush border membrane in A and basolateral membrane II in B.

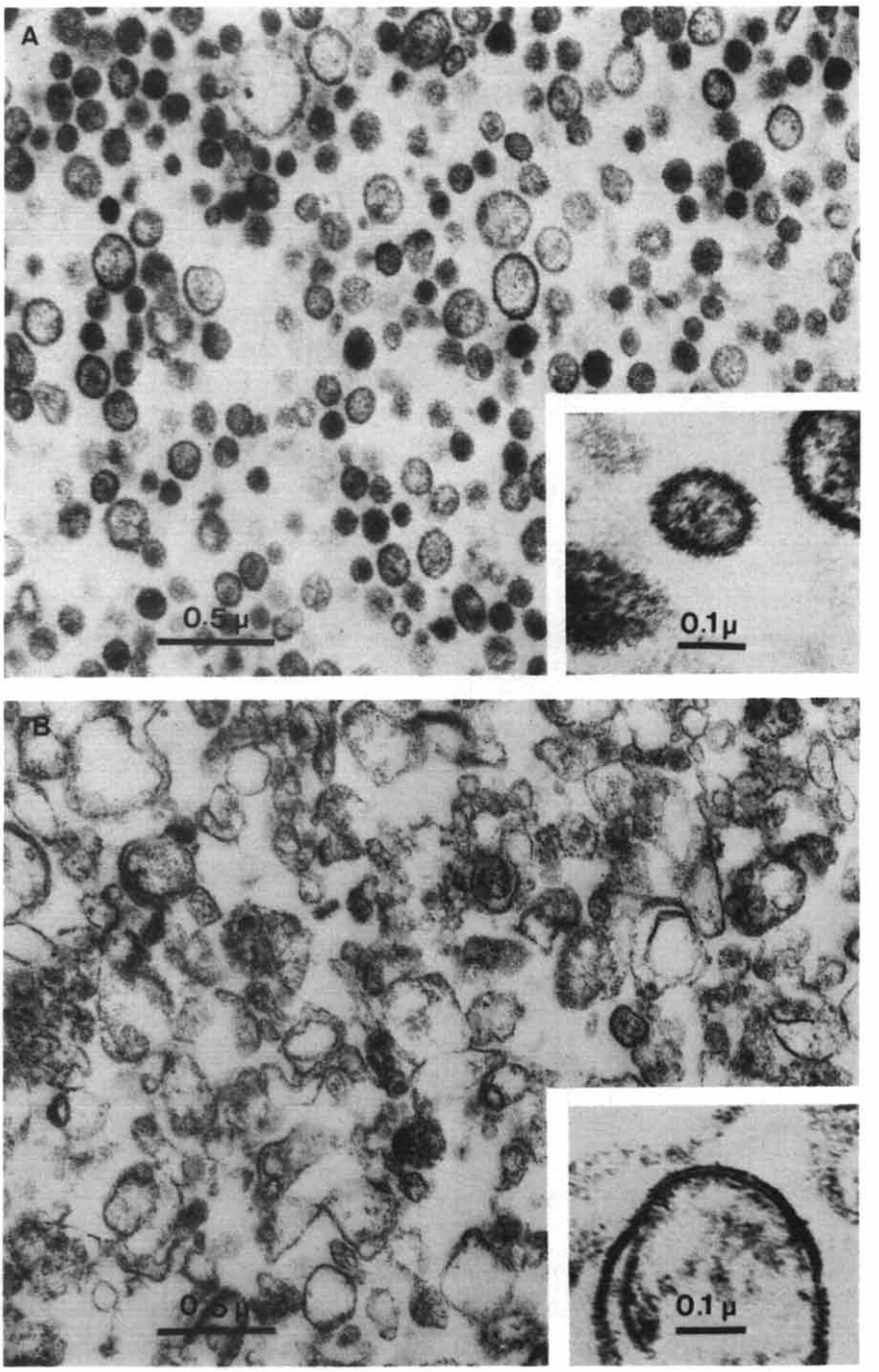


Fig. 2. Section through the pellet originating from brush border membrane in A and basolateral membrane I in B.

TABLE III

PRECIPITATION OF BRUSH BORDER AND BASOLATERAL MEMBRANES BY SPECIFIC AMINOPEPTIDASE N ANTIBODIES

For each enzyme the figures indicate the percentage of units found in the immunoprecipitate. The figures are the average of four independent assays for the brush border and basolateral II membranes and two assays for the basolateral I membrane. n.d., not determined.

Membrane	Aminopeptidase N	5′-Nucleotidase	$(Na^+ + K^+)$-ATPase
Basolateral I	25	18	15
Basolateral II	65	39	40
Brush border	95	95	—
Basolateral I + brush border *	60	n.d.	15

* Each membrane sample contained 50 units of aminopeptidase N.

contrast, only 25 and 65% of the aminopeptidase activity present in the basolateral membranes I and II, respectively, was detected in their immunoprecipitates and coprecipitation of 15—18 and 40% of the two specific basolateral markers, 5′-nucleotidase and $(Na^+ + K^+)$-ATPase, was also involved in that process. Furthermore, if an aliquot of brush border membrane were added to the basolateral membrane I and precipitation were performed as above, the brush border membrane was again totally recovered in the precipitate without, however, inducing a higher level of precipitation of the basolateral membrane markers.

(3) Solubilization by Emulphogen BC 7 20 and papain of the hydrolases present in the brush border and basolateral membranes

Table II shows that the basolateral membranes contain all the characteristic digestive enzymes of the brush border membrane. 5′-nucleotidase is also an enzyme shared by the two types of membrane, whereas $(Na^+ + K^+)$-ATPase seems to be strictly localized to the basolateral membranes.

All these enzymes could be solubilized from the three membrane fractions by Emulphogen BC 720 without loss of activity. Complete solubilization was achieved by an overnight incubation of the membranes (5 mg protein/ml) in 10 mM phosphate buffer (pH 7.4)/0.15 M NaCl and 2% Emulphogen.

Papain solubilization of the rabbit brush border hydrolases has already been performed in this laboratory [12]. The same conditions were used in these assays. To obtain some solubilization of alkaline phosphatase a 40 min hydrolysis had been chosen.

With the exception of the maltases, the basolateral enzymes were generally less sensitive to the papain action than those of the brush border membrane. Only 70—80% of aminopeptidase N and 50% of alkaline phosphatase were released from the basolateral membranes compared to 100% and 70%, respectively, from the brush border. About 30% of the 5′-nucleotidase was released from the three membrane types. During papain digestion the $(Na^+ + K^+)$-ATPase activity was lost.

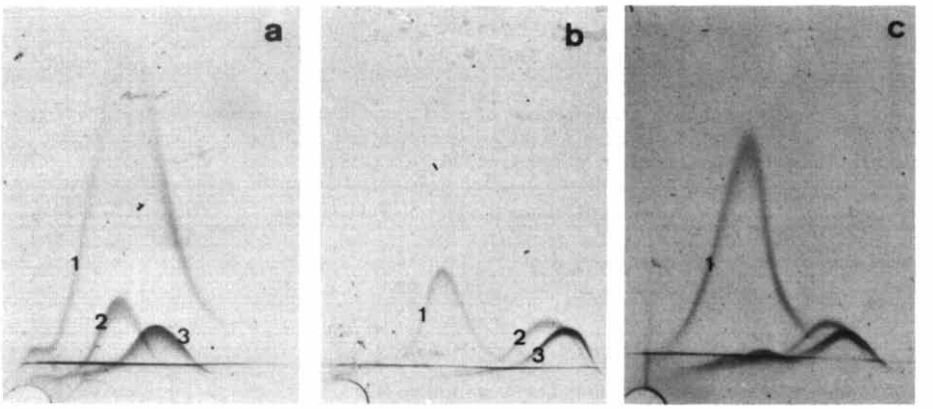


Fig. 3. Crossed immunoelectrophoresis of Emulphogen and papain solubilized proteins from basolateral membrane II. The gel used in the second dimension (from bottom to top) contained 500 μg/ml of immunoglobulins from guinea-pig sera raised against an Emulphogen extract of basolateral membrane II. Applied samples contained 4 units of aminopeptidase N: a, emulphogen-solubilized proteins (60 μg); b, papain solubilized proteins; c, mixture of 25% of a and 75% of b. The precipitates were revealed by staining with Coomassie Blue. The identified precipitates were: 1, enterocyte surface antigen; 2, aminopeptidase N; 3, sucrase-isomaltase complex.

(4) Characterization of proteins from basolateral and brush border membranes by crossed-immunoelectrophoresis

Crossed immunoelectrophoresis is a very convenient technique for resolving membrane proteins solubilized by neutral detergents [22], particularly in the case of brush border membrane hydrolases [6,23,24]. The technique was applied to the basolateral membranes II and I and the results are depicted in Figs. 3 and 4.

In Fig. 3, it can be seen that only three components were revealed in the emulphogen and papain extracts of basolateral membrane II by using immunoglobulins from guinea-pig sera raised against the Emulphogen extract. Two of them, peaks 2 and 3, were easily identified as the aminopeptidase N and the sucrase isomaltase complex, respectively. As expected the enzymes solubilized by papain migrated faster than those solubilized by emulphogen [18]. The material corresponding to the peak 1 was identical in both extracts as demonstrated in Fig. 3c where they were tested together and only one peak in position of peak 1 could be detected. The component of peak 1 was tentatively identified to the 'enterocyte surface antigen' characterized in this laboratory [6]. It was poorly solubilized by papain (comparison of Fig. 3a and 3b) and was in a form that had the same electrophoretic migration as the form solubilized by detergent.

Fig. 4a shows that essentially the same result was obtained in the case of the basolateral membrane I, except that a fourth protein precipitate appeared which has not yet been identified. Fig. 4b shows that the component giving the 4th immunoprecipitate also exists in basolateral membrane II. This component could not be detected in the brush border membrane under these conditions (Fig. 4c). However, it must be recalled that in order to see peaks 1, 2 and 3 the same amount of aminopeptidase units in the case of the three membranes

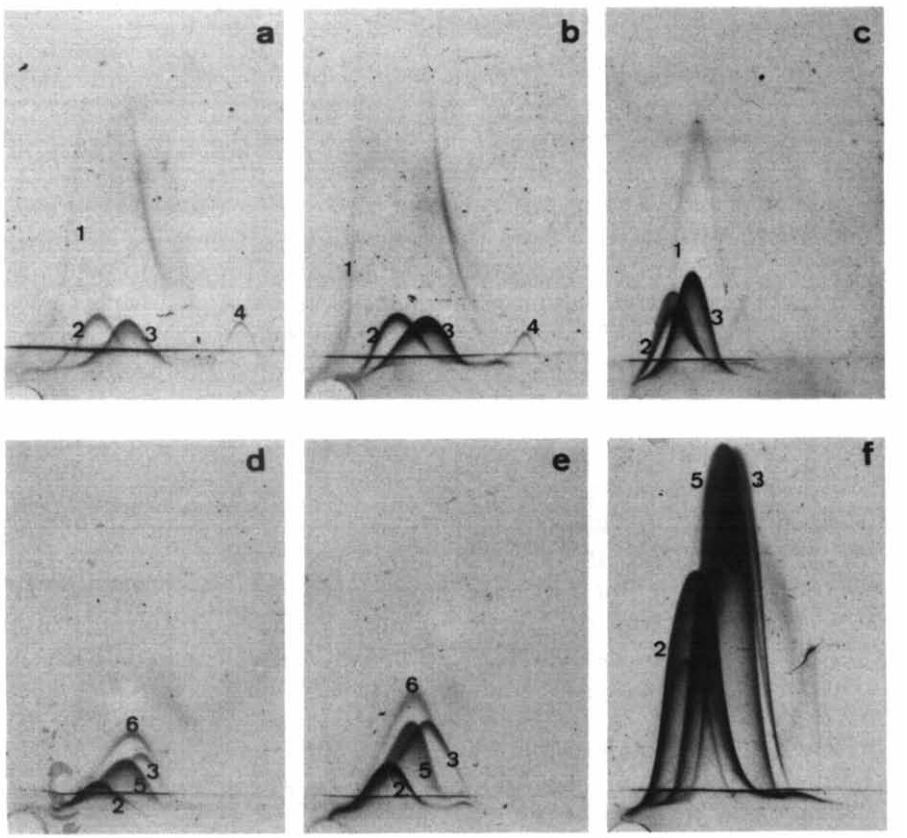


Fig. 4. Crossed immunoelectrophoresis of Emulphogen solubilized proteins from basolateral and brush border membranes. The second dimension gels contained immunoglobulins from guinea-pig sera raised against an Emulphogen extract of basolateral membrane I at a concentration of 300 μg/ml in a and 400 μg/ml in b and c, and immunoglobulins from guinea-pig sera raised against an emulphogen extract of brush border membrane at a concentration of 200 μg/ml in d and e and 500 μg/ml in f. The samples applied were: Emulphogen extract from basolateral membrane I (50 μg of proteins: 2.8 units of aminopeptidase N) in a and d; Emulphogen extract from basolateral membrane II (60 μg of proteins: 4 units of aminopeptidase N) in b and e; Emulphogen extract of brush border membrane: 4 units of aminopeptidase in c and 20 units of aminopeptidase in f. The precipitates were revealed after staining with Coomassie Blue. The precipitates identified were: 1, 2, 4, as in Fig. 3; 5, maltase; 6, aminopeptidase A.

was submitted to crossed immunoelectrophoresis. This means that to test the emulphogen extract of the brush border membrane with the serum raised against that of the basolateral membrane, the total amount of membrane proteins was at least 10 times lower then in the case of the basolateral membranes, which could explain the absence of peak 4 and the small size of peak 1 in Fig. 4c.

Figs. 4d, e and f show that the serum raised against the Emulphogen extract of the brush border membrane allowed the detection of two additional proteins in the basolateral membranes which were different from aminopeptidase N and sucrase-isomaltase. Peak 5 was attributed to a maltase. Aminopeptidase A activity was found in the immunoprecipitate 6.

Discussion

Comparative studies between different regions of a plasma membrane are more significant when carried out on different fractions prepared from the same starting material. With only slight modifications of the method described by Gratecos et al. [9] in the case of rat enterocytes, it has been possible to purify in good yield (15%) the brush border and the basolateral membranes from rabbit enterocytes starting from the same aliquot of mucosa. To obviate the risks of contamination of the basolateral membrane preparations by other intracellular membranes and plasma membranes from other cell types, several authors prefer to use isolated enterocytes as starting material [8,9]. However, Gratecos et al. [9] have shown that they obtained identical results when working either with isolated enterocytes or with a scraping of mucosa as is the case in the present work.

As in the case of the rat [9,19], the 5′-nucleotidase and $(Na^+ + K^+)$-ATPase, which are considered as basolateral membrane markers, are partitionated on sucrose gradient in two fractions (I and II) of density 1.14 and 1.16, which amount to 30% and 70% of the material, respectively. The enrichment factor for the two enzymes over the starting homogenate is identical in the two fractions and amounts to about 20-fold for 5′-nucleotidase and 10-fold for the $(Na^+ + K^+)$-ATPase. In the case of this latter enzyme, a loss of activity was always observed during the sucrose gradient centrifugation.

The enrichment factor for alkaline phosphatase and aminopeptidase N is approximately 18-fold in the brush border membrane preparations.

Considering the protein content (Table I) of the brush border and basolateral membranes prepared with about the same yield (calculated with specific markers), it must be pointed out that the two types of membrane must represent an equal proportion of the total plasma membrane of the cell.

At least 93% of the aminopeptidase N and alkaline phosphatase of the cell are localized to the brush border membrane (Table I and II) while the basolateral membrane contains about 7% of these enzymes. These hydrolases, which are present in both membrane preparations, are in total immunological homology as demonstrated by the crossed immunoelectrophoresis studies depicted in Figs. 3 and 4. Surprisingly, no antibody against specific basolateral membrane proteins has been induced when the emulphogen extracts of these membrane preparations have been injected as antigen.

As shown previously [21], the specific antibodies against aminopeptidase can quantitatively precipitate the brush border membrane (Table III). Considering the level of inhibition that they produce, these antibodies react with the aminopeptidase present in the basolateral membrane as well as with that of the brush border membrane. However complete precipitation of the aminopeptidase activity of the basolateral fractions could not be achieved: only 25% and 65%, respectively, were precipitated from fractions I and II. The specific markers of this membrane 5′-nucleotidase and $(Na^+ + K^+)$-ATPase coprecipitated in somewhat lower but very significant proportion (15 and 40%). The 5′-nucleotidase of the brush border fraction totally coprecipitated with the aminopeptidase. These results show that the presence of brush border hydrolases in the basolateral membrane preparations does not result from a contamination by

the brush border membrane. These enzymes are integrated in the basolateral membrane but their distribution is probably heterogeneous. Indeed, the difference in partial precipitation of the basolateral membrane subfractions 1 and II by anti-aminopeptidase antibodies emphasises their own heterogeneity although they contain an equal amount of aminopeptidase N (Table II). Studying the presence of endoplasmic reticulum marker enzymes in Golgi fractions, the Palade group obtained very similar results [25,26].

As shown in Table II, hydrolases which are major constituents of the brush border (alkaline phosphatase, aminopeptidases N and A, maltases) are all present in the basolateral membranes in amounts equal to about 5—7% of that present in the brush-border membrane. In contrast, lactase, which is in large amount only in the brush-border of suckling animals [27,28], is only three times more abundant in the brush border membrane of adult rabbits than in their basolateral membrane. The same result had been noted in the case of rat trehalase, another minor brush border hydrolase [9].

A strict relationship may well exist between the presence of brush border hydrolases in the basolateral membrane and the biogenesis of the brush border membrane. It is known that synthesis of the hydrolases begins in the very young cells of the crypts. Then the enzymes are incorporated in the undifferentiated plasma membrane which is closely related to the basolateral membrane. When differentiation is triggered, the brush border starts to grow and the level of the hydrolases increases tremendously in the brush border membrane [9,29, 30]. At least two mechanisms can be envisaged to explain this specific localization: (1) when the differentiation is induced the newly synthesized hydrolases are incorporated directly in the apical region of the cell; (2) the newly synthesized hydrolases are constantly incorporated in the basolateral membrane and when a basal level is reached, a segregation mechanism directs them towards the apical region of the plasma membrane, in other words, in the growing brush border.

The presence of the terminal web under the brush border and the observations reported in this paper in the case of the lactase and by Gratecos et al. [9] for the alkaline phosphatase in crypt and villous rat enterocytes seem to favour the second mechanism. It will be possible to choose between these two hypotheses by studying the biosynthesis of the aminopeptidase N, a study which is now under investigation in our laboratory. Indeed, according to the second mechanism the hydrolases located in the basolateral membrane of the villous cells would correspond to the brush border hydrolases in transit. Therefore, these enzymes will always represent the newly synthesized proteins.

In contrast, the first mechanism postulates that they have been synthesized in earlier stages of the differentiation. It would be of importance to determine the amount of lactase present in the basolateral and brush border membranes of suckling rabbits. This indeed would be a direct approach to answer the question of whether or not the level of hydrolase activities present in the basolateral membrane is constant, independent of that in the brush border membrane.

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