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STUDIES ON THE ARRANGEMENT OF AMINOPEPTIDASE AND ALKALINE PHOSPHATASE IN THE MICROVILLI OF ISOLATED BRUSH BORDER OF RAT KIDNEY

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SUMMARY

Brush-border fractions isolated from rat-kidney cortex were treated with papain and different concentrations of nonionic, cationic and anionic detergents. In this way significant differences in the solubility of aminopeptidase and alkaline phosphatase are noted, which can be related to their diverse organisation in the brush borders.

The aminopeptidase is localised on the surface of the microvillus matrix. It is released without a concomitant solubilization of alkaline phosphatase by incubation with papain and the nonionic detergent Triton X-100. The apparent molecular weights after removal with papain, anionic and nonionic detergents are 140000, 145000 and 168000, respectively.

The alkaline phosphatase is localised in the matrix of the microvilli and more strongly bound than the bulk of the protein. Its apparent molecular weights are dependent on the agents used for the disorganisation of the brush borders. After incubation with papain the lowest molecular weight is obtained.

INTRODUCTION

In order to elucidate the functions of plasma membranes of the apical pole of renal tubular cells in the transepithelial transport, we have described previously the enzymatic components of isolated brush borders[1], their surface structure[2], and the presence of a glucose binding site[3,4] which is probably involved in glucose transport. For investigation of enzyme-dependent transport mechanisms, methods had to be developed by which the brush borders could be disaggregated into their protein components, some of which are enzymes, for example the microvillus-bound aminopeptidase and alkaline phosphatase[1,5–10]. The isolated proteins can be used as antigens to produce specific antibodies. If the enzymes played a role in a transport system then the antibodies would be expected to plug the transport.

We therefore tried to localise and isolate the microvilli-bound enzymes aminopeptidase and alkaline phosphatase from isolated brush-border fractions. The brush-borders were disaggregated with neutral salt, papain, and increasing concentrations of nonionic, cationic and anionic detergents into small fragments until no

proteins could be sedimented by centrifugation. The activities of aminopeptidase and alkaline phosphatase and the amount of protein in the supernatants and sediments compared with that of the untreated brush-border fractions gave information about the degree of the disaggregation of the microvilli. The apparent molecular weights of the aminopeptidase- and alkaline phosphatase-containing fragments in the supernatants and sediments were also determined. Some speculation can be made about the localisation in the microvillus of the two enzymes which we studied on the basis of the ease of extraction from the brush borders and the molecular weight.

METHODS

Isolation of brush border

The isolation of the brush-border fraction was achieved as described by KINNE *et al.*[1]. The enrichment of brush-border membranes was controlled by the determination of marker enzymes such as alkaline phosphatase, aminopeptidase and glucose 6-phosphatase. In the brush-border fraction the specific activity of alkaline phosphatase was 12 times, and that of aminopeptidase 3.5 times higher than in the starting homogenate. The activity of the glucose 6-phosphatase was reduced. Other authors described increase of the alkaline phosphatase to the same level[9] during isolation of brush-border fractions.

Enzyme assays

Alkaline phosphatase (EC 3.1.3.1) was determined by the Merckotest^{R} method in the presence of 0.5 % bovine albumin.

The reaction mixture for the assay of aminopeptidase (EC 3.4.1.2) contained leucine-β-naphthylamide as substrate and was performed with a micromodification of the method described by GOLDBARG AND RUTENBURG[11].

The reaction mixture for the assay of glucose 6-phosphatase (EC 3.1.3.9) contained 0.06 M maleic acid (pH 6.7) and 0.02 M glucose 6-phosphate (sodium salt). To differentiate between specific and unspecific glucose 6-phosphatase the incubation was performed both in the absence and in the presence of 0.4 M D-glucose[12,13]. After 30 min of incubation at 37°, the samples were heated for 2 min in boiling water, chilled and centrifuged (2 min at 14000 rev./min, microsystem Eppendorf). The amount of P_i liberated was determined in aliquots of the supernatant by a modification of the method described by FISKE AND SUBBAROW[14].

Protein assay

The protein concentrations of the fractions were measured after precipitation of the protein by 10 % trichloroacetic acid in the cold and dissolution of the precipitate in 1 M NaOH according to LOWRY *et al.*[15]. As a standard protein bovine albumin was used.

Relative enzyme activity

This is defined as the ratio between the enzyme activity of aminopeptidase and alkaline phosphatase in the disorganised fraction and the activity of the same amount of untreated brush-border fraction.

The reason for changes of the relative activity of these enzymes is not due to differences in turnover numbers after the disorganisation of the brush borders. We could exclude this in experiments where 10 min after the addition of the detergent to the brush-border fractions the concentration of the detergent was reduced by dilution of the sample and the enzyme activities were determined instantaneously. Except after treatment with hexadecyltrimethylammonium bromide there was no change in activity compared with the untreated brush borders. A change in relative enzyme activity occurs only when the enzyme molecules have a longer contact with the detergents than during the centrifugation and dialysis procedures.

For the calculation of the distribution of the enzyme between supernatant and sediment the enzyme activities found therein were divided by the relative enzyme activity. These values were compared to the total content of aminopeptidase or alkaline phosphatase activity of the untreated brush-border fraction after correction of the protein loss.

Disaggregation of brush-border fractions

Freshly isolated brush-border fractions were shaken in LiCl solutions, nonionic, cationic and anionic detergents or digested with papain and then separated by centrifugation procedures. All centrifugations were done for 20 min at 35000 × *g* or 120 min at 100000 × *g*. The protein, aminopeptidase and alkaline phosphatase which did not sediment after centrifugation at 35000 × *g* was called the solubilized part of the brush-border fraction. The solubilized parts and the sediments were dialysed in Tris–NaCl buffer (0.15 M NaCl, 0.01 M tris(hydroxymethyl)aminomethane–HCl buffer (pH 7.6 at 20°)) for 18 h at 4° and protein and the activities of aminopeptidase and alkaline phosphatase were measured.

LiCl treatment

Brush-border fractions were suspended in sucrose–triethanolamine buffer (0.25 M sucrose, 0.01 M triethanolamine–HCl buffer (pH 7.6 at 20°)) containing 1.5 % (w/v) LiCl in a ratio 10:1 (mg LiCl per mg brush-border protein) for 10 min. Centrifugation and further treatment are described under METHODS.

Papain digestion

The effect of papain was investigated after the incubation of sucrose–triethanolamine buffer suspended brush borders with papain (1 mg protease per 27 mg brush-border protein) at 37°. The protease was activated by KCN (ref. 16). Centrifugation and further treatment are described under METHODS.

Treatment with detergents

To brush-border fractions suspended in sucrose–triethanolamine buffer the following surface active substances were added: nonionic: Triton X-100; cationic: hexadecyltrimethylammonium bromide; anionic: sodium deoxycholate and sodium dodecyl sulfate.

The concentrations applied are shown in Table I. After shaking for 10 min at 4° the fractions were centrifuged and treated as described under METHODS.

TABLE I

TREATMENT OF BRUSH-BORDER FRACTIONS WITH CATIONIC, NONIONIC AND ANIONIC DETERGENTS

The ratio of detergent to protein and the final concentration of the detergent in the solutions are shown.

Detergent	*Detergent/protein ratio (mg/mg)*	*Final detergent concn. (g/100 ml)*
Hexadecyltrimethyl-ammonium bromide	1:1	0.20
	2:1	0.40
	4:1	0.80
Triton X-100	1:1	0.15
	2:1	0.30
	4:1	0.60
Sodium deoxycholate	1:2	0.05
	1:1	0.10
	2:1	0.20
Sodium dodecyl sulfate	1:10	0.07
	1:3	0.21
	1:2	0.35
	1:1	0.70

Separation of fractionated brush-border membranes in polyacrylamide-gel disc electrophoresis

In order to discover if the brush-border fractions are disorganised in amino-peptidase and alkaline phosphatase containing fragments respectively, samples of the solubilized fractions were run on polyacrylamide gels. Electrophoresis was carried out on the polyacrylamide-gel system of REISFELD *et al.*[17]. Acrylamide concentration of the standard gels was 7.5 %. 50–100 μg of brush-border protein were layered into the gel column through the upper buffer. Electrophoresis was carried out at 2–4° and 4 mA per gel. Gels were stained over night with Coomassie blue and destained in 7 % acetic acid. Aminopeptidase and alkaline phosphatase were determined by incubating the gels with the substrates[18–20].

Molecular weight determination of aminopeptidase- and alkaline phosphatase-containing fragments in sodium dodecyl sulfate polyacrylamide-gel disc electrophoresis

The samples of the 100000 × *g* supernatants and sediments were shaken in sodium dodecyl sulfate–sucrose-triethanolamine-EDTA-dithiotreitol buffer (0.25 M sucrose, 0.01 M triethanolamine–HCl buffer (pH 7.6 at 20°C), 5 mM EDTA, 2 mM dithiotreitol, 2.5 mM sodium dodecyl sulfate) before electrophoresis was carried out. Unlike standard gels, this one contained 0.1 % sodium dodecyl sulfate. To all solutions for gel polymerisation therefore such a volume of 10 % sodium dodecyl sulfate was added, that the final concentration was 0.1 %. Upper and lower buffer: 0.1 % sodium dodecyl sulfate, 0.26 M glycine, 0.05 M tris(hydroxymethyl)amino-methane–HCl buffer (pH 8.3 at 20°). Electrophoresis was carried out as described

for the standard gels. Aminopeptidase and alkaline phosphatase were determined by incubating the gels with the appropriate substrates.

The following proteins with known molecular weights were used as references: ovalbumin, bovine serum albumin, aldolase, human γ-globulin and catalase.

A linear relationship between rates of migration and the logarithm of the molecular weights of proteins was found from MAIZEL and co-workers[21,22] by electrophoresis in polyacrylamide gels in the presence of sodium dodecyl sulfate.

Before electrophoresis the proteins were incubated in 1 % sodium dodecyl sulfate and mercaptoethanol. This concentration of sodium dodecyl sulfate when used in our studies produced a strong decrease of the enzymatic activity of the aminopeptidase and alkaline phosphatase. Neither enzyme can be localized according to its enzyme activity in the gel columns after electrophoresis. The use of only 0.1 % sodium dodecyl sulfate allows the enzymatic determinations of aminopeptidase and alkaline phosphatase in the gel column. We can, moreover, demonstrate a linear relationship between the logarithm of the molecular weights of reference proteins and their rates of migration, as in Fig. 1.

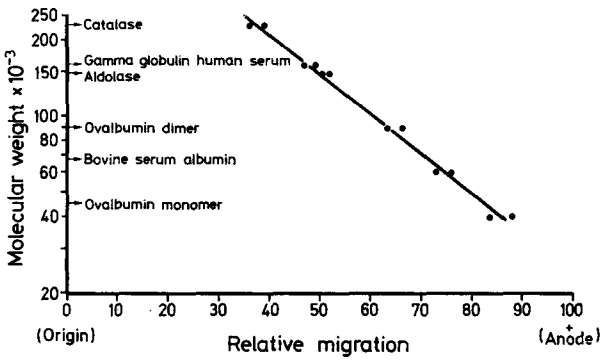

Fig. 1. Semilogarithmic relationship between molecular weight of the reference proteins and their relative speed of migration. Each point is the mean of two experiments.

Further, there is a good agreement between the molecular weight of the aminopeptidase- and alkaline phosphatase-containing fragments in the Triton X-100 supernatant determined by gel chromatography and sodium dodecyl sulfate polyacrylamide-gel disc electrophoresis. The equilibration of the samples in sodium dodecyl sulfate–sucrose–tris(hydroxymethyl)aminomethane–EDTA–dithiothreitol buffer prior to the electrophoretic run and the following electrophoresis in the sodium dodecyl sulfate-containing gel system have the disadvantage that untreated brush borders are separated into aminopeptidase and alkaline phosphatase containing fragments. The preceding treatment of brush-border membranes with detergents or papain leads to obvious alterations in the electrophoretic migration of the membrane fragments. These alterations are due to the specific actions of the used detergents and not to a fractionation during the electrophoretic procedures.

Gel filtration

Gel filtration was performed on Sephadex G-200 columns 0.9 cm × 140 cm, equilibrated with 0.01 M Tris–0.15 M NaCl buffer (pH 7.35). 0.4 ml of the 100000 × g

TABLE II

THE PERCENT RECOVERY OF PROTEIN, AMINOPEPTIDASE AND ALKALINE PHOSPHATASE IN THE 35000 × *g* SUPERNATANT OF KNOWN AMOUNTS (TOTAL) OF THESE SUBSTANCES IN THE UNTREATED BRUSH-BORDER FRACTION

The brush-border fraction was treated for 10 min with LiCl, detergents and papain. The relative activity is the ratio of the enzyme activity in the disorganised brush-border fractions to that of the same amount of the untreated preparation. The solubilization factor is the ratio of the percentage of enzyme solubilized to that of the protein solubilized. A ratio above 1 indicates the preferential release of enzyme relative to protein and *vice versa*. The means ± S.D. from 8 experiments are shown. HTAB, hexadecyltrimethylammonium bromide.

Solubilizing agent	*Concn.* (*g*/100 *ml*)	*Protein* (% *of total*)	*Aminopeptidase*			*Alkaline phosphatase*		
			% *of total*	*Relative enzyme activity*	*Solubilization factor*	% *of total*	*Relative enzyme activity*	*Solubilization factor*
HTAB	0.8	26.4 ± 1.4	0.3 ± 0.1	1.0 ± 0.1	0.02	1.1 ± 0.2	0.43 ± 0.1	0.03
LiCl	1.5	23.4 ± 1.6	4.6 ± 0.5	1.52 ± 0.4	0.20	2.1 ± 0.4	1.33 ± 0.5	0.06
Triton X-100	0.15	60.5 ± 8.2	62.7 ± 17	0.93 ± 0.1	1.04	13.1 ± 6.0	0.82 ± 0.3	0.22
	0.30	71.6 ± 6.3	81.8 ± 8.4	0.91 ± 0.1	1.14	20.6 ± 3.1	0.84 ± 0.2	0.29
	0.60	72.5 ± 6.1	97.6 ± 5.2	0.94 ± 0.1	1.35	39.4 ± 4.2	0.80 ± 0.2	0.54
Sodium deoxycholate	0.05	20.4 ± 9.8	6.4 ± 2.2	0.88 ± 0.2	0.31	5.8 ± 1.8	0.82 ± 0.2	0.28
	0.10	71.3 ± 3.2	92.3 ± 3.1	0.84 ± 0.2	1.30	54.2 ± 6.1	0.78 ± 0.1	0.76
	0.20	80.5 ± 5.8	97.7 ± 5.3	0.79 ± 0.2	1.21	83.0 ± 7.1	0.74 ± 0.2	1.03
Sodium dodecyl sulfate	0.07	63.0 ± 8.3	21.8 ± 6.9	1.10 ± 0.2	0.35	25.4 ± 4.4	0.89 ± 0.2	0.40
	0.21	100 ± 2.4	100 ± 6.3	1.49 ± 0.3	1.00	100 ± 1.3	0.86 ± 0.1	1.00
Papain		20.6 ± 6.4	88.2 ± 7.6	0.96 ± 0.3	4.28	7.0 ± 2.9	1.40 ± 0.2	0.34

supernatants of Triton X-100 disorganized brush borders were applied to the column and eluted with the same buffer.

MATERIALS

Ovalbumin, bovine serum albumin, aldolase and human serum globulin were obtained from Serva Heidelberg, catalase from Boehringer Mannheim. The analytical acrylamide-gel electrophoresis apparatus was purchased from Shandon Frankfurt.

RESULTS

LiCl treatment

Centrifugation of brush-border fractions in sucrose–triethanolamine buffer at 35000 × *g* caused a complete sedimentation of aminopeptidase and alkaline phosphatase; 89 % of the protein was spun down. Compared with this, after treatment with sucrose–triethanolamine buffer containing 1.5 % LiCl, only 77 % of the protein sedimented. Also, small activities of aminopeptidase and alkaline phosphatase were extracted from the microvilli, as seen in Table II. The low values of the solubilization factors indicate the preferential extraction of protein relative to each of the enzymes. When the resuspended sediment was run in polyacrylamide-

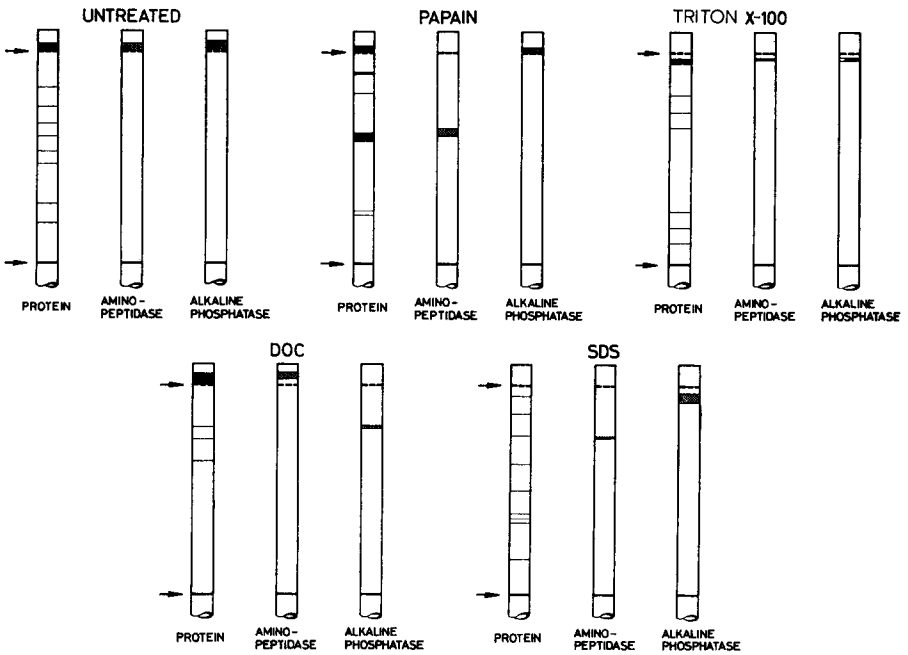

Fig. 2. Polyacrylamide-gel disc electrophoresis of solubilized brush-border fractions with either papain, or 0.30 % Triton X-100, or 0.20 % sodium deoxycholate (DOC), or 0.21 % sodium dodecyl sulfate (SDS). For comparison the polyacrylamide-gel electrophoretic pattern of an untreated brush-border fraction is also shown. The lines show the migrating protein fractions, whereas the dotted areas show a positive enzymatic reaction.

gel disc electrophoresis, no aminopeptidase- or alkaline phosphatase activity could be found in the gel with an average pore radius of 50 Å (Fig. 2). The enzymes are therefore still bound to fragments of larger size.

Hexadecyltrimethylammonium bromide treatment

Shaking brush-border fractions in hexadecyltrimethylammonium bromide solutions produced no solubilization of aminopeptidase- or alkaline phosphatase activities when the detergent was employed in concentrations of 0.2–0.4 %. Only small amounts of both enzymes could be detected in the supernatants when a 0.8 % hexadecyltrimethylammonium bromide solution was applied. The low solubilization factor shows a preferential extraction of protein from the fraction relative to aminopeptidase and alkaline phosphatase (Table II).

Some of the activity of the brush-border-bound alkaline phosphatase is lost during the incubation with hexadecyltrimethylammonium bromide for 10 minutes.

Treatment with papain

Nearly all the aminopeptidase remained in the 35000 × *g* supernatant when brush-border fractions were digested with papain, but alkaline phosphatase was spun down (Table II) and therefore still bound to microvilli fragments of large size. The high solubilization factor for aminopeptidase suggests that this enzyme is uniquely arranged in the microvillus.

In polyacrylamide-gel disc electrophoresis, aminopeptidase migrates into the separating gel, but not the alkaline phosphatase (Fig. 2).

Triton X-100 treatment

Incubation of brush-border fractions with increasing amounts of Triton X-100 per mg brush-border protein showed a concentration-dependent increase of aminopeptidase and alkaline phosphatase in 35000 × *g* supernatants (Table II). Aminopeptidase and protein were preferentially solubilized relative to alkaline phosphatase. The largest portion of the latter enzyme was sedimentable and therefore must be localised in larger microvilli fragments. An increase of Triton X-100 concentration from 0.6 to 1.2 % (not shown in Table II) extracted little additional alkaline phosphatase. The centrifugation of 0.3 % Triton X-100 disorganised brush borders at 100000 × *g* instead of 35000 × *g* showed no increase in sedimentability of aminopeptidase and alkaline phosphatase. Merely 10 % more protein sedimented. The specific activity of aminopeptidase in the supernatants after chromatography on Sephadex G-200 was 2.4 and 2.8 units and about 40-fold greater than in the brush-border fraction (two observations).

As can be seen in Fig. 2, aminopeptidase and alkaline phosphatase from 35000 × *g* supernatants have the same migration rates in polyacrylamide-gel disc electrophoresis. This result does not depend on one fragment in which both of the enzyme activities are localized but on two proteins with the same electrophoretic mobility in the standard gel system.

This interpretation is supported by results obtained by work with Sephadex G-200 column chromatography. In this latter system aminopeptidase- and alkaline phosphatase-containing fragments have a molecular weight of 174000–180000.

Actually, however, fragments containing both enzymes should have a much

larger molecular weight as was established in the sodium dodecyl sulfate polyacryl amide-gel disc electrophoresis study.

Sodium deoxycholate treatment

Protein, aminopeptidase and alkaline phosphatase were extracted from the microvilli depending on the concentrations of sodium deoxycholate, as can be seen in Table II. Aminopeptidase was solubilized from the microvilli in preference to protein and alkaline phosphatase.

The sodium deoxycholate concentrations which we tested were not sufficient to completely solubilize the brush borders. The centrifugation of 0.2 % sodium deoxycholate disorganized brush borders at 100000 × *g* instead of 35000 × *g* showed no increased sedimentation of alkaline phosphatase, but 6 % more of amino-peptidase and 11 % more of protein were spun down.

Aminopeptidase in 35000 × *g* supernatants remained in the spacer gel, while alkaline phosphatase migrated in polyacrylamide-gel disc electrophoresis. Therefore, both enzymes are not held in a common fragment after sodium deoxycholate treatment.

Sodium dodecyl sulfate treatment

Brush-border fractions are completely solubilized in sodium dodecyl sulfate concentrations higher than 0.21 %, as can be seen in Table II. The solubilization factors for aminopeptidase and alkaline phosphatase have the values of unity. Some of the dissolved enzymes and protein in the 35000 × *g* supernatants could be sedimented at 100000 × *g*. As shown in Fig. 3, the sedimentation of these substances is dependent on the sodium dodecyl sulfate concentration used for their solubilization. Increasing the sodium dodecyl sulfate concentration per mg brush-border protein disaggregated the microvilli into smaller and smaller fragments, which have a low tendency to sedimentation. The specific activity of amino-peptidase in the sediment after 0.21 % sodium dodecyl sulfate treatment is 0.42 unit and of alkaline phosphatase 6.8 units. In sediments after 0.35 % sodium dodecyl sulfate treatment,

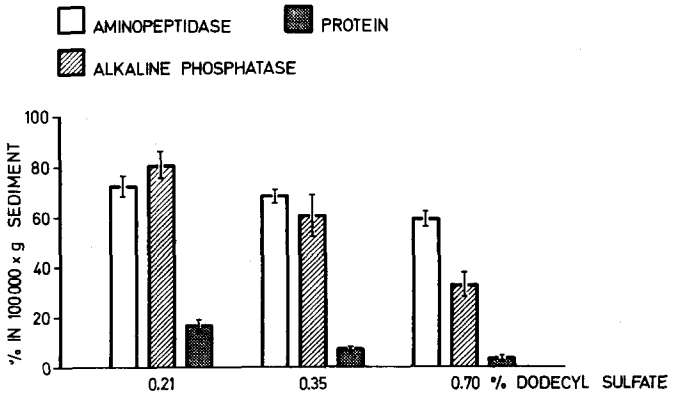

Fig. 3. Treatment of brush-border fragments with various concentrations of sodium dodecyl sulfate and subsequent centrifugation at 100000 × *g*. Shown are the recoveries of aminopeptidase, alkaline phosphatase and protein in the sediment expressed as a percent of the original solubilized fraction at 35000 × *g*. The bars show the mean ± S.D. of 5 experiments.

the alkaline phosphatase has a specific activity of 10.2 units while the activity of aminopeptidase is strongly decreased. The apparent molecular weight of the alkaline phosphatase found in these sediments is about 151000 ± 7000 by sodium dodecyl sulfate polyacrylamide-gel disc electrophoresis.

As shown in Fig. 2, aminopeptidase and alkaline phosphatase of 0.21 % sodium dodecyl sulfate solubilized brush-border fractions have different migration rates in disc electrophoresis. Brush borders are therefore disorganised by sodium dodecyl sulfate in fragments which contain either aminopeptidase or alkaline phosphatase.

Molecular weight determination in sodium dodecyl sulfate polyacrylamide-gel electrophoresis

The apparent molecular weights of the smallest aminopeptidase- or alkaline phosphatase-containing fragments after treatment of brush borders with the different detergents and papain are shown in Table III.

TABLE III

APPARENT MOLECULAR WEIGHT OF AMINOPEPTIDASE AND ALKALINE PHOSPHATASE IN SODIUM DODECYL SULFATE POLYACRYLAMIDE-GEL DISC ELECTROPHORESIS

Shown are the means from 4 experiments together with the S.D.

Treatment of brush-border fraction	*Molecular weight* × 1000	
	Aminopeptidase	*Alkaline phosphatase*
Untreated	166 ± 8	>230
0.30% Triton X-100, supernatant 100000 × *g*	168 ± 8	176 ± 10
0.20% sodium deoxycholate, supernatant 100000 × *g*	150 ± 6	164 ± 5
0.21% sodium dodecyl sulfate, supernatant 100000 × *g*	145 ± 7	160 ± 8
0.21% sodium dodecyl sulfate + 6 M urea, supernatant 100000 × *g*	145*	157*
Papain digestion, supernatant 35000 × *g*	140 ± 8	124 ± 4

* Mean from 2 experiments.

When untreated brush borders were electrophoresed on sodium dodecyl sulfate-containing gels, membrane fragments containing aminopeptidase as well as alkaline phosphatase were formed. The apparent molecular weight of the latter fragment was at least about 230000.

DISCUSSION

Earlier biochemical and electronmicroscopic studies[1,23] with brush-border fractions of rat kidney have shown that: (1) the microvilli are covered with a glycoprotein layer which contains no aminopeptidase or alkaline phosphatase and can be removed by digestion with trypsin; (2) after removal of the glycoprotein layer,

the matrix, studded with particles, can be seen; (3) these particles can be removed with papain.

After treatment with papain the aminopeptidase no longer migrates with the narrow brush-border zone in a linear sucrose density gradient[23].

In the present studies, the results of hexadecyltrimethylammonium bromide and LiCl treatments support the suggestion that an outer protein layer which contains no aminopeptidase and no alkaline phosphatase exists. Thus, after this treatment 14–17 % more of the protein but no aminopeptidase or alkaline phosphatase, were solubilized. Disorganisation with low concentrations of sodium dodecyl sulfate and sodium deoxycholate also showed preferential solubilization of proteins from the brush-border fraction.

The preferential separation of aminopeptidase relative to protein and alkaline phosphatase from the microvilli digested with papain, suggests that this enzyme is localised on the surface of the matrix. The comparison of dissolved protein after papain digestion to that after LiCl incubation demonstrates furthermore that the aminopeptidase might be only a small part of the brush-border protein. The idea that aminopeptidase is associated with the surface particles was gained from the electron-microscopic and density-gradient centrifugation studies. Further support can be obtained from the findings of the narrow range in apparent molecular weights after treatment with papain and anionic detergents. This narrow range suggests that aminopeptidase is localised in the microvillus in such a way that detergents and papain remove this activity either as pure enzyme or as a particle which is not further disaggregated by these treatments. KENNY *et al.*[24] have isolated this enzyme from rabbit renal brush-border epithelia with papain and found a molecular weight of 126000 ± 15000, which agrees with our estimation after aminopeptidase isolation with sodium dodecyl sulfate, sodium deoxycholate and papain. "Particle bound" aminopeptidase from pig kidney has a molecular weight of 280000 (refs. 25 and 26) and the complex consists of 10 subunits with a molecular weight of 30000 each; the complex could not be disorganised into subunits by sodium dodecyl sulfate[27].

The small extraction of alkaline phosphatase relative to protein and aminopeptidase after subjection of the brush borders to Triton X-100 indicates a difference in the organisation of the two enzymes within the microvilli. Although low concentrations of sodium dodecyl sulfate and Triton X-100 extract 60 % of the protein only a small amount of alkaline phosphatase is released from the brush-border fraction. This suggests that the enzyme is more strongly bound to the microvilli matrix than the bulk of the protein and is associated with proteins or carbohydrates which maintain the structural arrangement of the matrix as earlier discussed by BINKLEY *et al.*[28]. They suggested that the glycoprotein-containing matrix of the microvilli may be composed of enzymes interconnected by carbohydrate chains. Relatively water insoluble structural proteins of low molecular weights were found in plasma-membrane fractions of mammalian kidneys by FITZPATRICK *et al.*[12]. The association of alkaline phosphatase with such proteins also could explain our findings. The specific release of aminopeptidase relative to alkaline phosphatase by the incubation with papain and the small removal of alkaline phosphatase relative to the protein after disorganisation of the microvilli with Triton X-100 further indicate that relatively little of this latter enzyme is located on the surface of the matrix. The specific activity of the alkaline phosphatase in the 100000 × *g* sediment after

disorganisation of brush borders with 0.35 % sodium dodecyl sulfate is 5.3 units lower than the purified one described by MELANI *et al.*[29]. The apparent molecular weight of the sedimented enzyme therefore does not correlate with the real molecular weight of the alkaline phosphatase.

By treatment of the brush-border fractions with papain, small activities of alkaline phosphatase were measured in the 35000 × *g* supernatants. The apparent molecular weights were lower than after treatment with the anionic detergents. This result demands further investigation.

We are left with the interpretation of the above results, that aminopeptidase is localised on the surface of the microvillus matrix, may be particle-bound. The alkaline phosphatase is strongly bound to the microvillus matrix and can only be extracted by the disaggregation of the brush borders into small fragments which are not sedimentable by centrifugation at 35000 × *g*.

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