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Distributions of Post-Proline Cleaving Enzyme-, Converting Enzyme-, Trypsin- and Chymotrypsin-like Activities in Various Nephron Segments and in Brush-Border Membranes Isolated from Rat Kidney

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Distributions of post-proline cleaving enzyme-, converting enzyme-, chymotrypsin- and trypsin-like activities, were investigated in nephron segments and in brush-border membrane fraction isolated from rat kidney. These activities were found to be higher in the proximal tubule than in other segments, and were hardly detectable in the cortical collecting tubule. In the proximal tubule, trypsin-like activity showed the highest value in the pars convoluta, while the other peptidases showed their highest activities in the pars recta. Post-proline cleaving enzyme- and trypsin-like activities were also high in the glomerulus. In addition, post-proline cleaving enzyme- and converting enzyme-like activities were found in the brush-border membrane, while chymotrypsin- and trypsin-like activities were found in the cell component(s) other than the brush-border membrane. From these distributions of the peptidases, it is speculated that peptides are degraded both in the glomerulus and in the proximal tubule, and that in the proximal tubule the peptides are degraded both in the brush-border membrane and in other cell component(s).

Keywords——post-proline cleaving enzyme; converting enzyme; chymotrypsin; trypsin; aminopeptidase; brush-border membrane; nephron; kidney; microdissection; rat

Introduction

On the basis of microperfusion studies in the renal tubule, it has been reported that linear peptides such as angiotensin and bradykinin are degraded and reabsorbed mainly in the proximal tubule, and not at all in the distal nephron.[1] It has also been observed that the fluid collected by microperfusion in the proximal tubule contains breakdown products which are thought to be degraded by various peptidases, and it has been suggested that characteristic peptidases for these peptides exist in the proximal tubule.[1,2]

In earlier studies on the localizations of peptidases along the nephron, we previously determined the distributions of leucine aminopeptidase-, aminopeptidase A- and cystine aminopeptidase-like activities in various nephron segments isolated from the rat kidney.[3] However, it is known that peptidases other than the above aminopeptidases are involved in the degradation of the peptides in the nephron segments,[1,2] and their natures, functions and distributions remain to be further studied.

Figure 1 shows a diagrammatic representation of the breakdown of angiotensin, vasopressin, oxytocin and bradykinin by the peptidases. Trypsin and chymotrypsin cleave the Arg^2–Val^3 and Tyr^4–Ile^5 bonds of angiotensin, respectively.[4] Trypsin also cleaves the Arg^8–Gly^9 bond of vasopressin.[5] Chymotrypsin cleaves the Leu^8–Gly^9 bond of oxytocin, and the Phe^5–Ser^6 and Phe^8–Arg^9 bonds of bradykinin.[1a,5] Post-proline cleaving enzyme cleaves Pro^7–Arg^8 of vasopressin, Pro^7–Leu^8 of oxytocin, Pro^7–Phe^8 of angiotensin and Pro^3–Gly^4 as well as Pro^7–Phe^8 of bradykinin.[6] Converting enzyme, which converts angiotensin I into

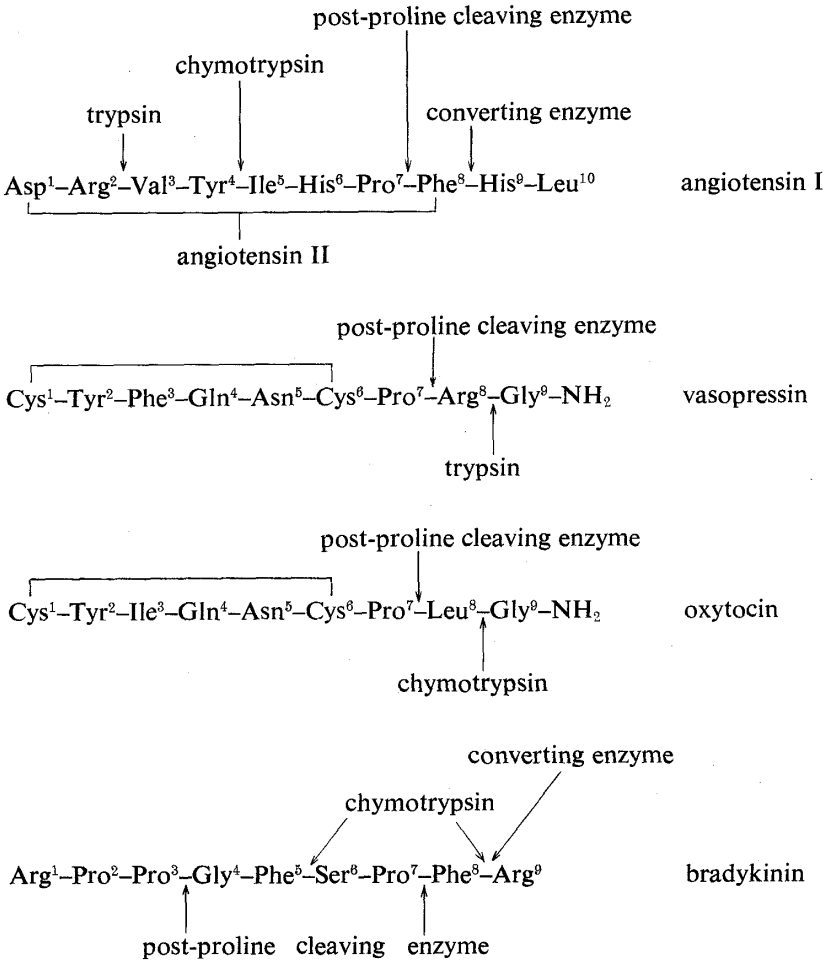

Fig. 1. A Hypothetical Scheme for the Breakdown of Angiotensins, Vasopressin, Oxytocin and Bradykinin by Various Peptidases

angiotensin II, is thought to be identical to kininase II, and cleaves the Phe^8–Arg^9 bond of bradykinin.[1a,7]

The kidney contains peptidases similar to trypsin and chymotrypsin; they may be called "trypsin-like enzyme(s)" and "chymotrypsin-like enzyme(s)" since they show some differences in biochemical characteristics from trypsin and chymotrypsin.[2,8] Though further investigations are needed to confirm that these trypsin- and chymotrypsin-like enzymes are involved in the degradation of peptides in the nephron, we nevertheless tried to assay the distributions of post-proline cleaving enzyme-, converting enzyme-, trypsin- and chymotrypsin-like activities in various nephron segments of the rat by developing new micromethods. Using artificial substrates, the activities of the peptidases degrading the above physiologically active peptides were investigated, and their activities were represented as U per length as well as U per protein content in order to compare the data with the above physiological results obtained by micropuncture techniques. In addition, using the brush-border membrane fraction isolated from the rat kidney, we investigated whether or not the individual peptidases studied in this paper as well as in the previous paper[3] are distributed in the brush-border membrane.

Experimental

Chemicals——Hyaluronidase (bovine testes, Type 1-S), *N*-succinyl-L-phenylalanine-*p*-nitroanilide and hippuryl-L-histidyl-L-leucine were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.), collagenase (type 1, CLS I)

from Worthington Biochemical Co. (Freehold, N.J., U.S.A.), 7-(*N*-benzoyl-D,L-argininamide)-4-methyl-coumarin, *N*-succinyl-glycylprolyl-4-methyl-coumarinyl amide and 4-methyl-aminocoumarin from the Protein Research Foundation (Osaka, Japan), and *p*-nitroaniline from Boehringer Mannheim (Mannheim, West Germany). All other reagents used were commercial products of the highest grade available. Male Wistar rats weighing 200 ± 20 g were obtained from Sankyo Labo Service Co. (Tokyo, Japan).

Preparation of Isolated Nephron Segments——Male Wistar rats were used for the experiments. Nephron segments were prepared with collagenase and hyaluronidase by the new microdissection method,[3] which is a modification of the methods of Imbert–Teboul *et al.*[9a] and of Sudo and Morel.[9b] The following five nephron segments were microdissected and used for analysis.[3] 1) Glomerulus in the superficial cortex (Glm). 2) Proximal convoluted tubule (PT1): PT1 was within 4 mm from the glomerulus. 3) The transitional portion (PT2) from the pars convoluta to the pars recta of the proximal tubule, which contained mainly the upper portion of the pars recta, about 1 mm. 4) The terminal portion of the pars recta of the proximal tubule (PT3), about 1 mm. 5) Cortical collecting tubule, about 1 mm. In the determination of enzyme activities per protein content, a large quantity of identical nephron segments was collected and homogenized, and then the protein contents[3] and the enzyme activities were measured.

Measurements of Enzyme Activities in the Isolated Nephron Segments——In this study, the following four enzyme activities were determined in the above nephron segments by means of microspectrophotometry and microfluorophotometry.

Chymotrypsin-like Activity——The activity was measured by a micromodification of the method of Nagel *et al.*,[8a] and by making use of diazotization of *p*-nitroaniline. *N*-Succinyl-L-phenylalanine-*p*-nitroanilide was used as the substrate, and the volume of the incubation medium[8a] was 35 μl; the protein content was about 7 μg. The incubation was carried out at 37 °C for 3 h. After incubation, 10 μl of 20% perchloric acid was added to stop the reaction, and the mixture was kept standing in ice for 30 min. After centrifugation at $10062 \times g$ for 10 min at 4 °C, 20 μl of the supernatant was transferred into another microtube, 3 μl of cold 1.6% sodium nitrite solution was added and the mixture was kept for 10 min at 4 °C. Five min after the addition of 3 μl of 4% ammonium sulfamate, 6 μl of 0.8% *N*-1-naphthyl-ethylenediamine in methanol was added. The whole was kept standing at 37 °C for 30 min in a dark room, then the absorbance was measured at 546 nm. The activity was calculated from a standard curve made by using *p*-nitroaniline.

Post-Proline Cleaving Enzyme- and Trypsin-like Activities——Post-proline cleaving enzyme-like activity was determined by a micromodification of the method of Yoshimoto *et al.*[6b] The composition of the incubation medium was as follows: 0.1 M phosphate buffer (pH 7.0), 0.5 mM *N*-succinyl-glycylprolyl-4-methyl-coumarinyl amide. The protein content was about 700 ng, and the volume was 30 μl. The incubation was carried out at 37 °C for 6 min.

Trypsin-like activity was determined by a micromodification of the method of Zimmerman *et al.*[10a] 7-(*N*-Benzoyl-D,L-argininamide)-4-methyl-coumarin was used as the substrate, and the volume of the incubation medium (pH 7.0)[10a] was 16 μl: the protein content was about 2 μg. The incubation was carried out at 37 °C for 2 h.

In the measurements of post-proline cleaving enzyme- and trypsin-like activities, the incubation was stopped by adding a solution of 10% Tween-20 in 1 M acetate buffer (pH 4.0): 10 μl in the case of post-proline cleaving enzyme-like activity and 5 μl in the case of trypsin-like activity. The relative fluorescence was measured at excitation and emission wavelengths of 370 and 440 nm, respectively. The activity was calculated from a standard curve made by using 4-methyl-aminocoumarin.

Converting Enzyme-like Activity——This enzyme activity was measured by modifying the method of Cushman and Cheung[7a] to increase the sensitivity, with a micromodification. The buffer was changed from 0.1 M phosphate buffer (pH 8.4) to 0.1 M borate buffer (pH 8.4). Hippuryl-L-histidyl-L-leucine was used as the substrate, and the volume of the incubation medium[7a] was 30 μl; the protein content was about 5 μg. The incubation was carried out at 37 °C for 3 h, and stopped by addition of 30 μl of 1 N HCl. The hippuric acid formed was extracted into 100 μl of ethyl acetate by mixing for 15 s at setting 5 in a Vortex mixer (Vortex-Genie, model K-500-G; Scientific Industries Inc., Bohemia, N.Y., U.S.A.). After centrifugation at $1700 \times g$ for 10 min at room temperature, a 50 μl aliquot of the ethyl acetate layer was transferred to another microtube, and evaporated. The hippuric acid formed was redissolved in 60 μl of water, and the absorbance was measured at 228 nm. The activity was calculated from a standard curve made by using hippuric acid.

Isolation of Brush-Border Membrane from Rat Kidney——In this experiment, 30 rats were used. Isolation of the brush-border membrane was carried out by modifying the methods of Knauf *et al.*[10b] and of Malathi *et al.*[10c] This method depends on preferential aggregation of subcellular structures other than brush-border membranes by Ca^{2+} ions. The kidney was exposed through a ventral incision under ether anesthesia, and perfused *via* the abdominal aorta with 0.9% NaCl solution. After perfusion, the kidney was removed and put into cold 0.9% NaCl solution. The kidney was sliced along the cortico-medullary axis, and the cortex was cut out and collected. The pieces of cortex were lysed in a hypotonic medium containing 60 mM sucrose, 4 mM Tris–H_2SO_4 at pH 7.4, and then gently homogenized with a glass-Teflon homogenizer. One volume of 100 mM $CaCl_2$ was added to 9 volumes of the homogenate: the final concentration of Ca^{2+} was 10 mM. After a 15 min incubation at 20 °C, the homogenate was centrifuged at $1500 \times g$ for 15 min at 4 °C, and the supernatant was recentrifuged at $27000 \times g$ for 15 min at 4 °C. After this centrifugation, a

white fluffy fraction located on the upper layer of the pellet was carefully collected, and washed twice by resuspension in isotonic medium containing 300 mM sucrose, 20 mM Tris–H_2SO_4 at pH 7.4 followed by centrifugation at 27000 × *g* for 15 min at 4 °C: this step was the most important of all the steps for purification of the brush-border membrane. The final white fluffy fraction contained the purified brush-border membrane.

For characterization of the brush-border membrane, the activities of the following enzymes were determined (the substrate used indicated in parenthesis): γ-glutamyl transpeptidase[11a] (γ-glutamyl-*p*-nitroanilide) as a marker for brush-border membranes, Na^+–K^+–ATPase[12a] (adenosine-5′-triphosphate) for baso-lateral membranes, cytochrome c-oxidase[12b] (ferrocytochrome c) for mitochondria, β-glucuronidase[12c] (4-methylumbelliferyl β-D-glucuronide) for lysosomes, catalase[11b] (hydrogen peroxide) for peroxisomes, glucose-6-phosphatase[11c] (glucose-6-phosphate) for microsomes and lactate dehydrogenase[11d] (pyruvate) for cytoplasm. In the determination of Na^+–K^+–ATPase activity, the filtrate obtained by filtering the homogenate through a triple gauze layer was used instead of the homogenate. In the case of Na^+–K^+–ATPase and glucose-6-phosphatase, the inorganic phosphorus released from the substrate was determined by the method of Youngburg and Youngburg.[12d] In addition to the peptidases in the present study, the activities of the aminopeptidases reported previously[3] were analyzed in the fractions obtained: these aminopeptidases include leucine aminopeptidase- (L-leucine β-naphthylamide), cystine aminopeptidase- (L-cystine di-β-naphthylamide) and aminopeptidase A- (α-L-aspartic acid β-naphthylamide) like enzymes, (the substrates are indicated in parenthesis). The protein content in each fraction was determined by the method of Lowry *et al.*[12e]

In the above assays, absorbance and fluorescence values were measured using a Hitachi-320 spectrophotometer (Hitachi Ltd., Tokyo, Japan) and an Aminco Fluoro-Colorimeter (American Instrument Co., Inc., Silver Spring, Md., U.S.A.), respectively. In microassays of enzymes, microcuvettes which were made for adaptation to the above machines were used.[3,12f]

Statistics——Results are given as means ± S.E. Statistical significance was assessed by means of Student's *t*-test: *p* values of less than 0.05 were considered significant.

Results

Post-proline cleaving enzyme-, converting enzyme-, chymotrypsin- and trypsin-like activities per protein content in various nephron segments are shown in Table I. According to the previous report,[3] the mean values of protein content per glomerulus or per length of tubular segment were as follows: Glm, 76 ng/Glm; PT1, 233 ng/mm; PT2, 282 ng/mm; PT3, 179 ng/mm; CCT, 100 ng/mm. From these values and the values in Table I, it is possible to calculate the activity per glomerulus or per length of tubular segment. Table II shows the values in various nephron segments.

The post-proline cleaving enzyme-like activity per protein content was relatively high in

TABLE I. Distributions of Post-Proline Cleaving Enzyme-, Converting Enzyme-, Chymotrypsin- and Trypsin-like Activities in Each Nephron Segment

	Post-proline cleaving enzyme-like activity	Converting enzyme-like activity	Chymotrypsin-like activity	Trypsin-like activity
Glm	16.0 ± 2.8	1.50 ± 0.19	0.0180 ± 0.0026	0.0250 ± 0.0058
PT1	10.6 ± 1.4	0.68 ± 0.20	0.0362 ± 0.0057	0.0431 ± 0.0092
PT2	16.0 ± 1.9	5.72 ± 0.73	0.0762 ± 0.0074	0.0136 ± 0.0022
PT3	24.0 ± 2.9	15.79 ± 4.05	0.0816 ± 0.0131	0.0096 ± 0.0020
CCT	1.0 ± 0.6	0.90 ± 0.30	0.0204 ± 0.0026	0.0101 ± 0.0020

The enzyme activity is represented in U/g·protein. Values each represent the mean ± S.E. The number of experiments in each nephron segment was 5 for each enzyme assay. Abbreviations of nephron segments are given in the experimental section. Statistics (*a*) $p<0.05$; *b*) $p<0.01$; *c*) $p<0.001$). Post-proline cleaving enzyme-like activity: Glm > CCT,[c] PT1 < PT3,[b] PT1 > CCT,[c] PT2 < PT3,[a] PT2 > CCT,[c] PT3 > CCT.[c] Converting enzyme-like activity: Glm > PT1,[a] Glm < PT2,[c] Glm < PT3,[b] PT1 < PT2,[c] PT1 < PT3,[b] PT2 < PT3,[a] PT2 > CCT,[c] PT3 > CCT.[b] Chymotrypsin-like activity, Glm < PT1,[a] Glm < PT2,[c] Glm < PT3,[b] PT1 < PT2,[b] PT1 < PT3,[a] PT1 > CCT,[b] PT2 > CCT,[c] PT3 > CCT.[b] Trypsin-like activity, Glm > PT3,[a] Glm > CCT,[a] PT1 > PT2,[a] PT1 > PT3,[b] PT1 > CCT.[b]

TABLE II. Distributions of Post-Proline Cleaving Enzyme-, Converting Enzyme-, Chymotrypsin- and Trypsin-like Activities per Glomerulus or per Tubular Length in Each Nephron Segment

	Post-proline cleaving enzyme-like activity	Converting enzyme-like activity	Chymotrypsin-like activity	Trypsin-like activity
Glm	1214	114	1.37	1.90
PT1	2468	161	8.43	10.03
PT2	4507	1614	21.49	3.84
PT3	4289	2828	14.61	1.73
CCT	100	90	2.04	1.01

The enzyme activity is represented in nU/Glm or mm of tubular length. Values were calculated from the activity per protein content (Table I) and from the protein content[3] per glomerulus or per length. Other details are as in Table I.

TABLE III. Distributions of Marker Enzymes in the Fractions of the Homogenate and the Brush-Border Membrane

Enzyme	Homogenate	Brush-border membrane	Relative activity
γ-Glutamyl transpeptidase	1972 ± 185	23578 ± 2959	11.96
Na^+–K^+–ATPase	(21.4 ± 1.9)	3.8 ± 1.6	(0.18)
Cytochrome c-oxidase	238 ± 22	6.3 ± 1.7	0.03
β-Glucuronidase	0.963 ± 0.045	0.138 ± 0.014	0.14
Catalase	175 ± 12	20 ± 2	0.11
Glucose-6-phosphatase	52.8 ± 1.7	21.0 ± 1.7	0.40
Lactate dehydrogenase	635 ± 25	23 ± 1	0.04

The number of experiments was 4, and the values are given as means $\pm$ S.E. The enzyme activity is represented in U/g·protein. In the measurements of Na^+–K^+–ATPase and glucose-6-phosphatase, one unit means 1 μmol of Pi hydrolyzed from the substrate per min. Relative activity was calculated by dividing the mean value in the brush-border membrane by the mean value in the homogenate. The Na^+–K^+–ATPase activity in parenthesis in the homogenate was determined by using a sample obtained by filtering the homogenate through a triple layer of gauze.

the glomerulus, and the proximal tubule showed a rise in activity from the pars convoluta to the pars recta [PT1 < PT3 ($p < 0.01$), PT2 < PT3 ($p < 0.05$)]. Activity in the cortical collecting tubule was hardly detectable. In terms of the activity per length, the proximal tubule showed a rise in activity from the pars convoluta to the pars recta.

The converting enzyme-like activity per protein content was very low in the pars convoluta of the proximal tubule and the cortical collecting tubule. The activity in the glomerulus was low, but slightly higher than that of the pars convoluta of the proximal tubule [Glm > PT1 ($p < 0.05$)]. Only the pars recta of the proximal tubule showed high activity: the terminal portion of the pars recta of the proximal tubule showed the highest activity [PT2 < PT3 ($p < 0.05$)]. In terms of activity per length, the same tendency was recognized. It was characteristic that the activity appeared shown mainly in the terminal portion of the pars recta of the proximal tubule.

As regards the chymotrypsin-like activity per protein content, the proximal tubule showed a rise in activity from the pars convoluta to the pars recta [PT1 < PT2 ($p < 0.05$), PT1 < PT3 ($p < 0.05$), PT2 $\fallingdotseq$ PT3 (N.S.)]. The activities in the glomerulus and the cortical collecting tubule were very low. In terms of activity per length, the proximal tubule showed a rise in activity from the pars convoluta to the upper portion of the pars recta, and a decline

TABLE IV. Distributions of Various Peptidases in the Fractions of the Homogenate and the Brush-Border Membrane

Enzyme	Homogenate	Brush-border membrane	Relative activity
Leucine aminopeptidase-like activity	97±5	1056±22	10.89
Aminopeptidase A-like activity	11.7±0.6	120.3±2.6	10.28
Cystine aminopeptidase-like activity	0.36±0.08	3.56±0.09	9.89
Post-proline cleaving enzyme-like activity	9.1±0.2	84.7±3.6	9.31
Converting enzyme-like activity	3.2±0.2	36.3±3.0	11.34
Chymotrypsin-like activity	0.086±0.006	0.096±0.004	1.12
Trypsin-like activity	0.0435±0.0015	0.0141±0.0006	0.32

The number of experiments was 4, and the values are given as means±S.E. The enzyme activity is represented in U/g·protein. Relative activity was calculated by dividing the mean value in the brush-border membrane by the mean value in the homogenate.

from the upper portion to the terminal portion of the pars recta.

The trypsin-like activity per protein content was relatively high in the glomerulus [Glm > PT3 ($p<0.05$)], and the proximal tubule showed a decline in activity from the pars convoluta to the pars recta [PT1 > PT2 ($p<0.05$), PT1 > PT3 ($p<0.01$)]: the highest activity was seen in the pars convoluta. The collecting tubule showed activity as low as the terminal portion of the pars recta of the proximal tubule. In terms of activity per length, the same tendency was recognized in the proximal tubule. The distribution of trypsin-like activity was remarkably different from the distributions of other enzymes studied in this paper.

Table III shows a comparison of the specific activities of the main marker enzymes in the homogenate and the brush-border membrane. There was a clear-cut rise in the activity of γ-glutamyl transpeptidase by a factor of 11.96 in the brush-border membrane in comparison to the homogenate. In contrast, cytochrome c-oxidase as a mitochondrial marker and Na^+–K^+–ATPase as a baso-lateral marker showed extremely low activity. This indicates that the brush-border membrane fraction was effectively separated from mitochondrial and contraluminal contaminants. The activities of β-glucuronidase as a marker enzyme of lysosomes, catalase (peroxisomes), glucose-6-phosphatase (microsomes) and lactate dehydrogenase (cytoplasm) were all very low. Thus, the brush-border membrane fraction was sufficiently pure to use in the following study.

Table IV compares the specific activities of the peptidases in the homogenate and the brush-border membrane. Leucine aminopeptidase-, aminopeptidase A- and cystine aminopeptidase-like activities, whose distributions in the various nephron segments were previously reported,[3] showed clear-cut rises in activity by a factor of nearly 10 in the brush-border membrane in comparison to the homogenate. Post-proline cleaving enzyme- and converting enzyme-like activities showed similar rises in the brush-border membrane. Accordingly, these peptidases were considered to be present in the brush-border membrane. In contrast, chymotrypsin- and trypsin-like activities showed relatively low activities (factors of 1.12 and 0.32, respectively) in the brush-border membrane in comparison to the homogenate. This result indicates that these two peptidases are not present in the brush-border membrane.

Discussion

In the previous paper,[3] seven portions along the nephron were investigated to determine the distributions of aminopeptidases, while only five portions (excluding the ascending limb of Henle's loop and the distal convoluted tubule) were investigated in the present study. This was because it is more difficult to collect the above two segments than other segments in the rat, and relatively more nephron pieces are needed in order to measure the peptidase activities in the present study, as compared with the peptidases in the previous study.[3] For example, for the measurement of chymotrypsin-like activity, more than 14 μg of protein content was required (blank and sample). This amount (14 μg) of protein corresponds to about 180 mm length in the case of the ascending limbs of Henle's loop.[3] It was difficult to collect such large amounts of the ascending limb of Henle's loop and the distal convoluted tubule in the rat, and also to measure the exact length of large amounts of tubular pieces. Therefore, in this study, only the above five portions were used, and the enzyme activity was represented directly in U per protein content and indirectly in U per glomerulus or per length.

As mentioned in the previous study,[3] we have used the term "-like activity" or "-like enzyme" in naming the enzymes studied in this study, because the specificities for the substrates employed were not necessarily characteristic of the parent peptidases.[1,2,6-8]

As to the purification of the brush-border membrane from the rat kidney, γ-glutamyl transpeptidase as the main marker enzyme showed a rise in activity by a factor of 11.96 in the brush-border membrane in comparison to the homogenate. This indicates that relatively high purification of the brush-border membrane was achieved from the rat kidney. However, it may not be sufficient to evaluate the degree of purity only from this result. Accordingly, the authors consider that the other means including histological studies should be used for further evaluation of the purity of the brush-border membrane.

The distributions of the peptidases studied in this paper can be summarized as follows. 1) High levels of post-proline cleaving enzyme- and trypsin-like activities were found in the glomerulus. 2) The highest activity in the pars convoluta of the proximal tubule was that of trypsin-like enzyme. 3) In the pars recta of the proximal tubule, post-proline cleaving enzyme-, converting enzyme- and chymotrypsin-like activities were high. 4) The activities of the above-mentioned peptidases were all very low in the cortical collecting tubule. 5) In the brush-border membrane fraction, post-proline cleaving enzyme- and converting enzyme-like activities were higher by a factor of nearly 10 as compared to the homogenate. The activities of the peptidases reported previously,[3] including leucine aminopeptidase-, aminopeptidase A- and cystine aminopeptidase-like activities, were also higher by a factor of nearly 10. 6) In the brush-border membrane fraction, chymotrypsin- and trypsin-like activities were relatively low (factors of 1.12 and 0.32, respectively).

Concerning the distribution of peptidases in the brush-border membrane, it was reported that converting enzyme was a component of the brush-border membrane,[7b] as well as the aminopeptidases reported previously,[3] including leucine aminopeptidase and aminopeptidase A activities.[13] Our results are consistent with these reports. In addition, in the present study, post-proline cleaving enzyme- and cystine aminopeptidase-like activities were newly determined to be present in the brush-border membrane. In contrast, it is speculated that chymotrypsin- and trypsin-like activities are present in cell component(s) other than the brush-border membrane. Differences in the distribution patterns of post-proline cleaving enzyme- and converting enzyme-activities in the three portions of the proximal tubule (PT1, PT2, and PT3) were also apparent.

Maunsbach[14a] reported that differences existed in cell structure, particularly concerning the morphological structure of the brush-border membrane, among the three portions of the proximal tubule (PT1, PT2, PT3) in his histological study of the nephron. Accordingly, the

differences in the distributions of the above-mentioned peptidases of the brush-border membrane of the proximal tubule may reflect both quantitative and qualititative heterogeneity ("intra-nephron heterogeneity")[3,14] of the brush-border membrane of the proximal tubule. As regards the trypsin-like activities, which may be distributed in a cell component(s) other than the brush-border membrane, it is not surprising that the pattern of distribution differed from the distributions of other peptidases. In contrast to the distribution of trypsin-like activity, the distribution of chymotrypsin-like activity showed a pattern similar to those of the peptidases of the brush-border membrane even though the chymotrypsin-like activity was also considered to be present in a cell component(s) other than the brush-border membrane. Thus, it seems likely that functional heterogeneity in the degradation of peptides, as well as in the cell components, exists along the nephron. Further studies on the locations of the chymotrypsin- and trypsin-like activities should be of interest.

In a microperfusion study on the degradation of peptides in the renal proximal tubule of the rat, Silbernagl and Völkl[15] reported that degradation products from the microperfused peptide were accumulated transiently in the tubular fluid and reabsorbed subsequently in the forms of small peptides and amino acids. It seems likely that the above peptidases in the brush-border membrane are related to the degradation of the peptide. However, it remains to be investigated whether large peptides are reabsorbed into the tubular cells, and whether the reabsorbed peptides are directly degraded in the cells by peptidases locating intracellularly. If these problems can be resolved, the role of the chymotrypsin- and trypsin-like activities should become clearer.

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