Activities of Aromatic L-Amino Acid Decarboxylase with L-Dopa as Substrate in Brush-Border- and Basolateral Membranes and Cytoplasm Obtained from Rat Renal Cortex

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Abstract—Activities of aromatic L-amino acid decarboxylase (AADC) with L-dopa as its substrate were determined in the plasma membranes and other cellular components isolated from rat renal cortex. The cytoplasm contained a high level of AADC activity, but much lower levels were found in the brush-border- and basolateral membranes. The main site involved in the formation of dopamine from L-dopa was considered to be the cytoplasm, with participation of neither the brush-border- nor the basolateral membranes.

Aromatic L-amino acid decarboxylase (AADC; EC 4.1.1.28) is an enzyme which decarboxylates L-dopa and L-5-hydroxytryptophan into dopamine and serotonin, respectively (1), and it occurs in several organ tissues (2). In the kidney, it has been reported that this AADC localizes in the renal proximal and distal tubules (3), and that dopamine is formed from plasma L-dopa in the kidney and is excreted into the urine (4, 5). Three sites can be proposed as possible renal cellular site(s) where the decarboxylation of L-dopa to dopamine occurs, if the concerned nephron segment is considered to be restricted to the proximal tubules: brush-border membranes, basolateral membranes and other cellular components.

In order to investigate the proximal tubular site(s) involved in the decarboxylation of L-dopa to dopamine, we prepared brush-border- and basolateral membranes and subcellular components from the rat kidney cortex, and studied the AADC activities with a substrate of L-dopa in the prepared fractions.

Male Wistar rats weighing 200–250 g were used. Under ether-anesthesia, the kidneys were exposed through a ventral incision, and perfused via the abdominal aorta with 0.9% saline, and removed. The kidneys were sliced along the cortico-medullary axis, and the pieces of cortex were cut out and collected.

For rough subcellular fractionations, the pieces of renal cortex were homogenized (1:9, w/v) in solution A, which consisted of 0.25 M sucrose, 1 mM ethylenediamine tetra-acetic acid disodium (EDTA·Na₂), 0.1 mM α -toluenesulfonyl fluoride (TSF), a protease inhibitor, and 10 mM Tris-HCl (pH 7.5), by a glass/Teflon Potter homogenizer. The homogenate was then centrifuged, in turn, at 1,000×g for 10 min, 10,000×g for 30 min and 100,000×g for 60 min. The sediments obtained were resuspended in solution A.

Brush-border- and basolateral membranes were isolated by methods employing calcium precipitation and Percoll density gradient centrifugation (6, 7). Every solution used for the above isolation contained 0.1 mM TSF to protect AADC against proteases.

For characterization of each fraction, the activities of the following enzymes were determined as reported previously (8): leucine aminopeptidase as a marker for brush-border membranes, Na⁺-K⁺-ATPase for basolateral membranes, cytochrome *c*-oxidase for mitochondria, β -D-glucuronidase for lysosomes, uricase for peroxisomes, glucose-6-phosphatase for microsomes and lactate dehydrogenase for the cytoplasm. Protein amount was determined by the method of Kresze (9), a procedure which is an improvement of the method of Lowry et al. (10).

AADC activity was determined by modifying the method of Nagatsu et al. (11). The preincubation medium consisted of 30 mM NaH_2PO_4 - Na_2HPO_4 buffer (pH 7.2), 0.3 mM $\text{EDTA}\cdot\text{Na}_2$, 0.17 mM ascorbic acid, 0.01 mM pyridoxal phosphate, 0.1 mM pargyline hydrochloride and 0.1 mM TSF. The medium in volume of 485 μl was put into a reaction vial and then preincubated at 37°C for 2 min. After adding 10 μl of sample and 5 μl of 40 mM L-dopa to the medium, the mixture was then incubated at 37°C for 30 min. The final concentration of L-dopa in this mixture was 0.4 mM: this concentration was approximately 4 times the K_m value (0.0935 ± 0.0240 mM, mean $\pm$ S.D.) which was determined using the homogenate from the rat renal cortex as the enzyme source. After the incubation, 400 μl of 0.2 N perchloric acid and 20 μl of 2 $\mu\text{g}/\text{ml}$ dihydroxy-benzylamine hydrobromide were added, which, respectively, stopped the enzymatic reaction and served as the internal standard for the HPLC method described below. Treatment of this aliquot and

the HPLC determination was done according to the method of Riggan and Kissinger (12). HPLC conditions employed in this study were as follows: isocratic elution; mobile phase, aqueous solution consisting of 30 mg/l sodium octyl sulfate, 2 mM $\text{EDTA}\cdot\text{Na}_2$, 0.1 M chloroacetic acid (pH 3.0, adjusted by crystalline sodium hydroxide); flow rate, 1 ml/min; detection, voltammetry (L-ECD-6A ECD voltammetry detector; Shimadzu Co., Kyoto, Japan; detector potential was set at 0.8 V versus the Ag/AgCl electrode); column, Shim-pack CLC-ODS (6 mm $\times$ 150 mm, 5 μm ; Shimadzu Co.); column temperature, 30°C.

Table 1 shows the specific activities of the main marker enzymes and AADC in the homogenate and the roughly-separated subcellular fractions. The supernatant fraction obtained by centrifugation at $100,000 \times g$ for 60 min contained the highest activity of lactate dehydrogenase and relatively lower activities of the other marker enzymes as compared to the other fractions. In this fraction, the highest AADC activity was also observed as com-

Table 1. Specific activities of marker enzymes and AADC in the homogenate and the subcellular fractions obtained from the rat renal cortex

Enzyme	Homogenate	Sediment 1	Sediment 2	Sediment 3	Supernatant
Leucine aminopeptidase	54.8 $\pm$ 3.6 (1.00)	57.6 $\pm$ 4.6 (1.05)	181.0 $\pm$ 10.9 (3.30)	229.8 $\pm$ 30.4 (4.19)	16.2 $\pm$ 2.5 (0.30)
Na^+ - K^+ -ATPase	43.8 $\pm$ 10.8 (1.00)	80.4 $\pm$ 24.3 (1.84)	142.6 $\pm$ 26.1 (3.26)	147.7 $\pm$ 45.7 (3.37)	0.1 $\pm$ 5.2 (0.002)
Uricase	46.4 $\pm$ 9.8 (1.00)	70.8 $\pm$ 20.6 (1.53)	139.3 $\pm$ 34.8 (3.00)	104.3 $\pm$ 19.6 (2.25)	32.7 $\pm$ 10.8 (0.70)
Glucose-6-phosphatase	79.2 $\pm$ 8.5 (1.00)	109.5 $\pm$ 22.2 (1.38)	171.8 $\pm$ 19.1 (2.17)	277.2 $\pm$ 55.4 (3.50)	45.7 $\pm$ 11.5 (0.58)
Lactate dehydrogenase	646 $\pm$ 6 (1.00)	254 $\pm$ 32 (0.39)	181 $\pm$ 34 (0.28)	159 $\pm$ 28 (0.25)	2385 $\pm$ 416 (3.69)
β -D-Glucuronidase	1.01 $\pm$ 0.15 (1.00)	1.49 $\pm$ 0.20 (1.48)	2.10 $\pm$ 0.19 (2.08)	0.64 $\pm$ 0.08 (0.63)	0.78 $\pm$ 0.20 (0.77)
Cytochrome c-oxidase	818 $\pm$ 166 (1.00)	901 $\pm$ 77 (1.10)	1655 $\pm$ 181 (2.02)	232 $\pm$ 44 (0.28)	3.7 $\pm$ 19.4 (0.005)
AADC	6.02 $\pm$ 1.07 (1.00)	2.68 $\pm$ 0.17 (0.45)	4.00 $\pm$ 1.59 (0.66)	4.08 $\pm$ 0.71 (0.68)	29.68 $\pm$ 3.96 (4.93)

Abbreviations: AADC, aromatic L-amino acid decarboxylase; Sediment 1, sediment obtained by centrifugation at $1,000 \times g$ for 10 min; Sediment 2, sediment obtained by centrifugation at $10,000 \times g$ for 30 min; Sediment 3, sediment obtained by centrifugation at $100,000 \times g$ for 60 min; Supernatant, supernatant obtained by centrifugation at $100,000 \times g$ for 60 min. The number of experiments was 6, and values are given as means $\pm$ S.D. The enzyme activity is given as U/g protein. In the measurements of Na^+ - K^+ -ATPase and glucose-phosphatase, one unit means 1 μmole of Pi hydrolyzed from the substrate per min. Relative activities, which are shown in parentheses, were calculated by dividing the mean value in each fraction by the mean value in the homogenate.

pared with the other fractions. The AADC activities in fractions other than the supernatant were found to be as low as that in the homogenate. Table 2 shows a comparison of the specific activities of the marker enzymes and AADC in the homogenate, brush-border membranes and basolateral membranes. There was a clear-cut rise in the activity of leucine aminopeptidase by a factor of 13.77 in the brush-border membranes and also a clear-cut rise in the activity of $\text{Na}^+\text{-K}^+\text{-ATPase}$ by a factor of 8.85 in the basolateral membranes. In contrast, the other marker enzymes showed relatively lower activities in the two membranes. The activities of lactate dehydrogenase, a cytoplasmic marker, were extremely low in the two membranes. This indicated that the two membrane fractions were effectively separated from the other cellular components, especially from the cytoplasm. The activities of AADC were very markedly lower in the two membranes as compared with that in the supernatant fraction.

In this study, using the fractions obtained from the renal cortex, intracellular localization of AADC was investigated. Although the renal cortex is composed of the glomeruli, proximal tubules and other distally located tubules (cortical distal tubules and collecting

tubules), most of the tubular segments are generally known to be proximal tubules. Accordingly, our results might almost reflect the enzymatic activities distributed in the proximal tubules.

When the site of the dopamine synthesis was considered to be mainly on the proximal tubules, the basolateral and brush-border membranes were the first "gates" in relation to the access process of L-dopa as a substrate for AADC. Our study denoted that the AADC activities with L-dopa as its substrate were distributed most highly in the cytoplasm, and lower amounts were found in the basolateral and brush-border membranes obtained from the rat renal cortex. Although the low AADC activities in the two membranes were considered to be due to contamination by the cytoplasm, the possibility that AADC may locate in the membranes could not be excluded. However, even this enzyme does exist in these membranes, the intraluminal formation of dopamine from L-dopa filtered through the glomeruli was speculated to be relatively lower in comparison to that of the cytoplasm.

Above all, it should be noted that the AADC activity was the highest in the cytoplasm. From this finding, it is speculated that dopamine is formed from L-dopa by AADC in the

Table 2. Specific activities of marker enzymes and AADC in the homogenate, brush-border membranes and basolateral membranes obtained from the rat renal cortex

Enzyme	Homogenate	Brush-border membranes	Basolateral membranes
Leucine aminopeptidase	42.3±6.0 (1.00)	582.6±49.8 (13.77)	98.1±11.1 (2.32)
$\text{Na}^+\text{-K}^+\text{-ATPase}$	48.0±8.1 (1.00)	0.9±17.3 (0.02)	425.0±17.6 (8.85)
Uricase	43.8±5.2 (1.00)	33.0±7.0 (0.75)	77.4±19.7 (1.77)
Glucose-6-phosphatase	67.7±19.0 (1.00)	48.4±11.2 (0.71)	376.4±109.6 (5.56)
Lactate dehydrogenase	635.7±169.0 (1.00)	33.6±10.8 (0.05)	116.7±11.3 (0.18)
$\beta\text{-D-Glucuronidase}$	1.41±0.15 (1.00)	0.16±0.02 (0.11)	0.38±0.10 (0.27)
Cytochrome c-oxidase	715.5±86.6 (1.00)	78.6±38.3 (0.11)	85.8±41.5 (0.12)
AADC	5.58±0.90 (1.00)	2.05±0.34 (0.37)	0.77±0.13 (0.14)

Explanations are as in Table 1.

cytoplasm in the proximal tubular cells, that the intracellularly formed dopamine is released to the peritubular and/or intraluminal fluids, and that dopamine released locally in the kidney plays multiple physiological roles (13–16). As to the urinary excreted dopamine, there have been no reports that dopamine has any physiological functions at the luminal site. Therefore, dopamine that was formed intraluminally and/or released into the lumen might be excreted into the urinary fluid without exerting any physiological functions, whereas the intraluminally reabsorbed dopamine may have a possible role in some functions. The dopamine which was finally released to the peritubular fluid might play the most important role in the physiological functions of the kidney.

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