Deficiency of membrane-bound dipeptidyl aminopeptidase IV in a certain rat strain

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9 June 1986

Summary. The activity of membrane-bound dipeptidyl aminopeptidase IV (EC 3.4.14.5) was found to be markedly reduced in the Fischer 344 rat strain compared with that in the Wistar strain. Analysis of membrane proteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Ouchterlony immunodiffusion revealed the specific absence of this enzyme molecule in the Fischer 344 strain.
Key words. Dipeptidyl aminopeptidase IV; enzyme deficiency; membrane-bound peptidase; Fischer 344 rat; Wistar rat.

Dipeptidyl aminopeptidase IV (DAP IV), first reported by Hopsu-Havu and Glenner[1], releases dipeptides with a penultimate prolyl residue from the N-terminal sequence of oligopeptides. The preferential removal of the N-terminal X-Pro by the enzyme suggested that DAP IV participates in the catabolism of biologically active peptides[2–6] and/or the post-translational processing of peptides[7]. On the other hand, clinical studies on the enzyme showed that the enzyme activity in human sera was increased in patients with hepatitis and decreased in patients with gastric cancer[8] and rheumatoid arthritis[9]. However, little is known about the precise pathophysiological role of this enzyme. It has been reported that the DAP IV is associated with a variety of cell membranes[10]. In the course of our studies on membrane-bound peptidases, a deficiency of DAP IV in membranes of Fischer 344 rats was found. In the present study, we first compared the level of four peptidase activities in various tissue membranes and urine including DAP IV between Wistar and Fischer 344 rats, and then the membrane proteins were analyzed by electrophoresis and immunodiffusion.

Materials and methods. Adult male rats of the Fischer 344 strain were obtained from Charles River Japan Inc., Kanagawa, and those of the Wistar strain from the Shizuoka Agricultural Cooperative Association, Shizuoka. Membranes were solubilized as described previously[11]. A brief description of the procedure is given below. The quickly removed organs were homogenized in 8 vols of ice-cold 0.25 M sucrose. The homogenate was centrifuged at 10,000 × g for 10 min and then the supernatant was further centrifuged at 105,000 × g for 1 h. The sedimented membranes were homogenized in 8 vols (per g original tissue) of a buffer containing 10 mM Tris-HCl (pH 8.0) and 1 M KCl, and then centrifuged as above. The KCl-washed membranes were homogenized in 8 vols of a buffer containing 10 mM Tris-HCl (pH 8.0), 0.1 M NaCl and 0.5% (w/v) Brij 35. The Brij-35 washed membranes were collected by centrifugation as above, and then solubilized with a buffer containing 10 mM Tris-HCl (pH 8.0), 25 mM NaCl and 1% (w/v) Nonidet P-40. After centrifugation as above, the solubilized supernatants were retained.

The 4 types of peptidases were assayed essentially as described previously[11]. DAP IV was assayed with 1 mM Gly-Pro-pNA·Tos. Aminopeptides was assayed with 1 mM Ala-pNA. Neutral endopeptidase was indirectly assayed with 1 mM Suc-$(Ala)_3$-pNA as substrate, in the presence of excess porcine kidney aminopeptidase M. γ-Glutamyl transpeptidase (γ-GTP) activity was determined with γ-Glu-pNA as substrate as described by Satoh et al.[12]. All assays were conducted at 37 °C and the changes in absorbance at 410 nm were measured. One enzyme unit was defined as the amount of the particular enzyme required for the formation of 1 μmol of pNA per min. Specific activities were expressed as units per mg solubilized protein. The protein content was determined by the method of Lowry et al.[13] with bovine serum albumin as the standard, in the presence of 2% sodium dodecyl sulfate (SDS).

The proteins of the Wistar and Fischer 344 rat kidney membranes were analyzed by SDS-polyacrylamide gel electrophoresis. Solubilized membranes (100 μg protein) were precipitated with acetone containing 0.05 N HCl and then subjected to polyacrylamide gel (10%) electrophoresis according to Laemmli[14]. After the run, the gel was stained for proteins with Coomassie brilliant blue G-250.

DAP IV was purified from male Wistar rat kidney according to the method of Fukasawa et al.[15]. Antiserum against DAP IV was raised in rabbits by injecting 1.6 mg of the purified enzyme, mixed with an equal volume of Freund's complete adjuvant, s.c. twice with an interval of 2 weeks. The rabbits were bled 2 weeks after the last injection. Immunodiffusion was performed overnight at 37 °C in 1.2% agarose and 1 M NaCl.

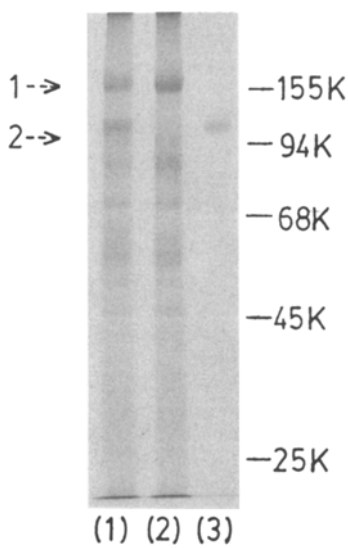

Figure 1. SDS-Polyacrylamide gel electrophoresis of the solubilized membranes of rat kidneys. Lane 1, solubilized membranes of Wistar rat kidney; lane 2, solubilized membranes of Fischer 344 rat kidney; and lane 3, purified DAP IV from Wistar rat kidney (10 μg protein). The molecular weight markers, indicated on the right, were *E. coli* RNA polymerase β' subunit (Mr. 155K), phosphorylase *a* (Mr. 94K), bovine serum albumin (Mr. 68K), ovalbumin (Mr. 45K) and chymotrypsinogen (Mr. 25K).

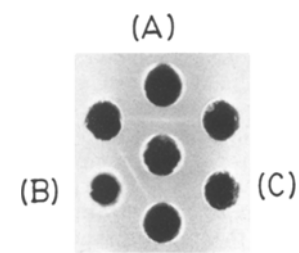

Figure 2. Immunodiffusion analysis of rat kidney membranes on an Ouchterlony plate. The center well contained 20 μl of the antiserum against the purified DAP IV. The outer wells contained: (A) 20 μl of the purified DAP IV (16 μg protein); (B) Wistar rat kidney solubilized membranes (72 μg protein); and (C) Fischer 344 rat kidney solubilized membranes (72 μg protein).

Comparison of the level of four peptidase activities in various solubilized membranes and urines of Wistar and Fischer 344 rats

	DAP IV		Aminopeptidase		Neutral endopeptidase		γ-GTP	
	Wistar	F344	Wistar	F344	Wistar	F344	Wistar	F344
Kidney	1.407	0.002	0.505	0.512	0.026	0.029	9.173	9.015
	±0.113	±0.001	±0.039	±0.035	±0.005	±0.005	±0.175	±0.243
Intestine	0.201	< 0.001	0.203	0.201	0.011	0.012	0.633	0.654
	±0.025		±0.007	±0.006	±0.002	±0.001	±0.043	±0.054
Lung	0.480	< 0.001	0.072	0.067	0.008	0.008	0.287	0.301
	±0.043		±0.007	±0.005	±0.001	±0.001	±0.009	±0.012
Liver	0.166	< 0.001	0.057	0.060	0.003	0.003	0.273	0.267
	±0.008		±0.004	±0.003	±0.001	±0.001	±0.015	±0.019
Submaxillary gland	0.103	< 0.001	0.117	0.106	0.004	0.004	0.143	0.158
	±0.006		±0.005	±0.004	±0.002	±0.002	±0.014	±0.013
Pancreas	0.063	< 0.001	0.071	0.065	0.003	0.003	0.962	1.053
	±0.009		±0.005	±0.004	±0.001	±0.001	±0.043	±0.038
Brain	0.029	< 0.001	0.044	0.048	0.002	0.002	0.283	0.294
	±0.002		±0.004	±0.003	±0.001	±0.001	±0.012	±0.009
Urine	0.014	< 0.001	0.006	0.010	< 0.001	< 0.001	0.178	0.205
	±0.002		±0.002	±0.003			±0.019	±0.020

The values are the means ± SE for the specific activities (units/mg protein) of the enzymes from three rats.

Results and discussion. As can be seen in the table, the specific activities of aminopeptidase, neutral endopeptidase and γ-GTP in the tissue membranes and urines tested were almost equal in the Wistar and Fischer 344 rat strains. On the other hand, negligible activity of DAP IV was seen in the Fischer 344 rat strain. The proteins of kidney membranes from the two strains showed similar patterns except for one protein band, which corresponded to a molecular weight of about 100,000 as shown in figure 1. This protein only existed in the Wistar rat membrane, and its mobility completely corresponded with that of the subunit of purified DAP IV. The largest protein, with a molecular weight of about 160,000, which is indicated by dotted arrow 1, corresponded to the subunit of aminopeptidase M[10]. The protein with a molecular weight of 92,000, indicated by dotted arrow 2, was neutral endopeptidase, which we purified previously[11]. These results suggested that the deficiency of DAP IV activity in the Fischer 344 strains is due to the lack of this enzyme protein in the membrane.

Recently, a deficiency of the activity of a mouse kidney brush border metalloendopeptidase called meprin was reported[16]. Certain inbred strains showed enzyme activities that were less than 5% of the normal level. Immunological experiments on meprin showed that an inactive form of the enzyme is present in the deficient mouse kidney[17]. However, as shown in figure 2, the solubilized protein from Fischer 344 rat kidney did not show any cross-reaction with the anti-DAP IV antiserum. This result indicates that an altered form of the DAP IV molecule is not present in the Fischer 344 membrane. There are several possible reasons for the deficiency of DAP IV in the Fischer 344 strain. The most likely explanations are as follows: 1) the absence of translatable mRNA because of a gene deficiency or a post-transcriptional processing disorder, or 2) an increase in the intracellular degradation of DAP IV because of an abnormal molecule. The Fischer 344 rat strain may provide a valuable tool for elucidating the precise role of DAP IV in the physiological state.

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0014-4754/87/040400-02$1.50 + 0.20/0