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Immunochemical identity of dipeptidyl aminopeptidase IV from pig serum, liver, submaxillary gland and kidney

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Summary. The enzymes which were extracted by autodigestion from the microsomal fractions of the pig kidney, liver and submaxillary gland and from the serum showed an immunochemical identity by a double immunodiffusion test. But the kidney enzyme had a different pI-value from the pI-values of the enzymes of other organs.

Dipeptidyl aminopeptidase IV, discovered and designated as glycyl-proline-2-naphtylamidase by Hopsu-Havu and Glenner[2], liberates N-terminal glycyl-L-proline from either glycyl-L-proline-2-naphtylamide or peptides. It was purified from the rat liver[3], pig kidney[4-7], human submaxillary gland[8] and lamb kidney[9]. The tissue distribution of the enzyme in the rat was examined histochemically[10]. The clinical study of the enzyme showed that the enzyme activities in the human sera were increased in patients with hepatitis and decreased in patients with gastric cancer[11] and rheumatoid arthritis[12]. In our previous report[7], we showed that the enzyme in the kidney was a glycoprotein containing 18.3% of carbohydrates and had a serine residue at the N-terminal position. In order to compare the chemical nature of the enzyme in several organs of the pig, an immunochemical study has been performed using the antibodies prepared against the enzyme purified from the pig kidney in the rabbit.

Materials and methods. Fresh pig kidney, liver, submaxillary gland, and blood were obtained from a slaughterhouse. They were stored at − 80 °C. The enzyme of the kidney was purified according to the method described in our previous report[7] and used as antigen. Antibodies against the enzyme were prepared in a male rabbit (2 kg) by injecting the antigen (2.0 mg of protein dissolved in 1.0 ml of saline) intracutaneously as a mixture with an equal volume of Freund's complete adjuvant 4 times at weekly intervals. The rabbit was completely bled and the serum was collected 7 days after the last injection. The equivalent point between the antiserum and the highly purified enzyme was determinded by the method of Kabat and Meyer[13]. The homogenates of the kidney, liver and submaxillary gland were prepared in 9 vol. of 0.25 M sucrose using an Ultra Turrax homogenizer, and the microsomal fraction of each homogenate was prepared by the method of Hogeboom[14]. Solubilization of the enzyme from each microsomal fraction was effected by autolysis[4], and the solubilized enzymes from these 3 organs were separated by ammonium sulfate fractionation (55–85%). The enzyme in the serum was also separated by ammonium sulfate fractionation (55–85%) and was further purified by DEAE-cellulose and sephadex G-200 column chromatographies by the previously reported procedures[7]. The enzyme activity was assayed by the photometric method of Nagatsu et al.[15], using Gly-Pro-p-nitroanilide tosylate (Gly-Pro-pNA) as substrate. 1 unit of enzyme activity was defined as the amount of enzyme catalyzing the formation of 1 µmole of p-nitroaniline per min at 37 °C.

The effect of antiserum on the enzyme activity was studied. 30–100 ng of the enzyme protein were mixed with 0.25 ml of antiserum and incubated for 45 min at 37 °C, then the enzyme activities were measured. The antiserum was also checked for enzyme activity, and the value was substracted from the total activities as a blank. Double immunodiffusion analysis and immunoelectrophoresis were performed by the method of Ouchterlony[16] and Scheidegger[17], respectively. Isoelectric focusing in polyacrylamide gel was carried out by the thin-layer slab gel technique[18]. Equal volumes of 2 kinds of Ampholine with different pH ranges

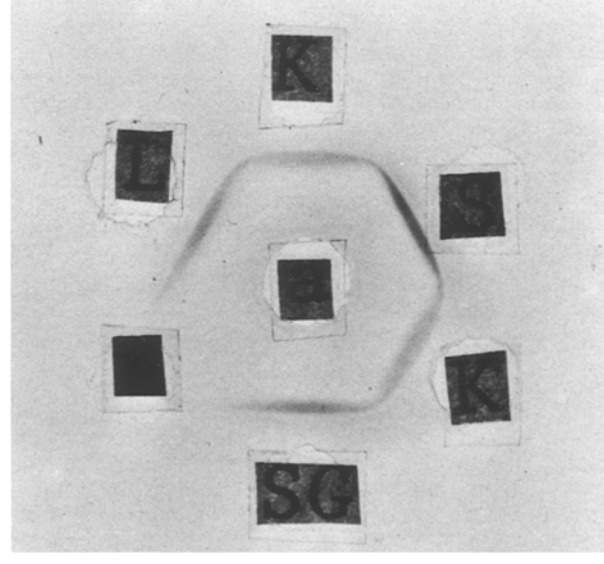

Fig. 1. Double immunodiffusion analysis of partially purified dipeptidyl aminopeptidase IV from the pig several organs. The center well (a) contained 20 µl of the antiserum to the kidney enzyme. The outer wells contained 20 µl of the partially purified enzyme. K: the kidney enzyme (10 µg); L: the liver enzyme (15 µg); SG: the submaxillary enzyme (15 µg); S: the serum enzyme (150 µg); B: blank, 0.01 M Tris-HCl buffer, pH 7.4.

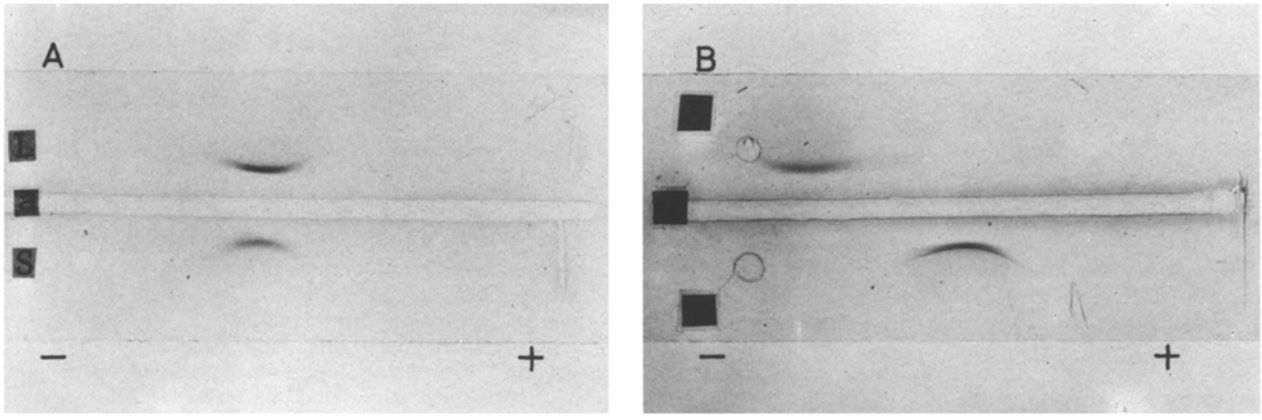

Fig. 2. Immunoelectrophoresis of partially purified dipeptidyl aminopeptidase IV from the pig several organs. K: the kidney enzyme (10 µg); L: the liver enzyme (15 µg); S: the serum enzyme (150 µg). Electrophoresis was run for 1 h at 6 mA/gel (2.5×7.5 cm) in 20 mM sodium barbital buffer, pH 8.6. And then 0.1 ml of the antiserum to the kidney enzyme was added to the channel (a). The plate was allowed to develop for 24 h at 37 °C.

(pH ranges, 3.5–5.0 and 5.0–7.0) were mixed, and the electrofocusing was performed for 4 h at 5 °C. The voltage was gradually raised from 40 to 80 V/cm. The position of the enzyme in the gel was located by measuring the enzyme activity in each gel piece of 5 mm width after cutting the gel. Protein was measured by the method of Lowry et al.[19], using bovine serum albumin as standard.

Results and discussion. Dipeptidyl aminopeptidase IV activities were found in the kidney, liver, submaxillary gland and serum, and the units of the activities per g wet weight were estimated to be 19.5, 4.70, 6.98 and 0.05, respectively. The enzyme activity was localized in the microsomal fraction in these 3 organs. The enzymes solubilized from the microsomal fraction of these 3 organs by autodigestion at pH 3.8 were separated by ammonium sulfate fractionation (55–85%). The specific activities of the partially purified enzymes from the kidney, liver and submaxillary gland were 3.7, 0.26 and 0.25 U/mg protein, respectively. The specific activity of the enzyme isolated from the serum was 0.05 U/mg protein.

The antibody concentration in the antiserum was calculated to be 3.1 mg/ml from the equivalent point. The antiserum did not show any inhibition on the enzyme activity even in an excess amount.

By double immunodiffusion analysis a single precipitin line was formed between antiserum and the enzyme from the liver, submaxillary gland, kidney and serum, and the 4 precipitin lines were completely confluent without any spur formation (figure 1). This result suggests the immunological identity of the 4 enzymes in these different organs. The pictures of immunoelectrophoreses are shown in figure 2. The precipitin arcs of the enzymes from the liver, submaxillary gland and serum were located in the same position (figure 2, A), whereas the arc of the kidney enzyme was located in a different position (figure 2, B). This result indicates that the enzymes in the liver, serum and submaxillary gland possess more acidic charges than the enzyme in the kidney. This possibility was further supported by the results obtained by isoelectric focusing. By measuring the pH-value and the enzyme activity in the solution extracted from each position of the gel, the pI-values of the liver, the submaxillary and the serum enzyme were determined to be 4.2, whereas the pI-value of the kidney enzyme was 5.2.

These results may suggest that the kidney enzyme is specific as compared with the liver, the submaxillary and the serum enzyme. However, since the kidney enzyme was proved to be a glycoprotein containing sialic acid[7], the difference in pI of the kidney and the liver/submaxillary enzyme is perhaps only related to a higher content in sialic acid in the liver/submaxillary enzyme, as a result of a postsynthetic modification which is not specific for any organ. This problem remains for further investigation.

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