Tissue-specific regulation of dipeptidyl peptidase IV expression during development*

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The patterns of dipeptidyl peptidase (DPP) IV activity and protein amount in different rat organs during development were compared. In order to elucidate the molecular basis for these patterns, total RNA was isolated from lung and kidney at different stages of development and analysed by Northern-blot hybridization using an oligonucleotide derived from the DPP IV cDNA sequence. This oligonucleotide hybridized to two distinct mRNAs of approx. 3.2 and 4.8 kb respectively. During kidney development, the pattern for DPP IV mRNA paralleled that of DPP IV activity and protein amount, suggesting that, in kidney, the expression of DPP IV is primarily controlled at the transcriptional level. In contrast, the magnitude of DPP IV activity during lung development compared with that of DPP IV mRNA in lung suggests that post-transcriptional mechanisms are involved in regulating the expression of DPP IV in lung. Organ-specific regulation of DPP IV expression may provide a useful model for further comparative studies of transcriptional and post-transcriptional mechanisms of DPP IV expression within the same organism.

INTRODUCTION

Dipeptidyl peptidase IV (DPP IV, EC 3.4.14.5) was first described in rat liver and pig kidney (Hopsu-Havu & Glenner, 1966; Hopsu-Havu & Sarimo, 1967) as an integral plasma-membrane protein (McNair *et al.*, 1979; Hartel *et al.*, 1989). It is widely distributed in mammalian tissues, with the highest activity in the kidney and the intestinal brush-border membrane. Characterized as a serine-type proteinase, DPP IV preferentially cleaves *N*-terminal dipeptides from polypeptides containing proline as the penultimate amino acid, as found in proteins such as the blood-pressure regulatory substance P, the fibrin α -chain or the natural analgaesic casomorphine 5 [for a review, see Heymann & Mentlein, 1984]. This enzyme was also shown to be involved in the interaction of hepatocytes or BHK (baby-hamster kidney) cells with the extracellular matrix (Hanski *et al.*, 1985; Reutter *et al.*, 1989), in particular by its binding to collagen, which was suggested as a possible substrate because of its high content of Gly-Pro sequences (Hopsu-Havu & Ekfors, 1969; Kenny, 1977).

Recently, the primary structure of DPP IV has been deduced from its cDNA, and the *N*-terminal signal sequence has been identified as the membrane-anchoring domain (Hong & Doyle, 1987; Ogata *et al.*, 1989).

Previous experiments on the regulation of DPP IV expression in different rat organs suggested the regulation of DPP IV expression to take place at the transcriptional level (Hong *et al.*, 1989).

Our results have led us to the conclusion that this is not true for all rat organs, since, by contrast, in some organs, post-transcriptional factors appear to play a role in the regulation of DPP IV expression. Furthermore, we were able to demonstrate the existence of two distinct RNA bands in Northern blots hybridized with an oligonucleotide derived from the DPP IV cDNA sequence (Ogata *et al.*, 1989).

MATERIALS AND METHODS

Chemicals and reagents used in this study included guanidinium isothiocyanate from Fluka, Buchs, Switzerland, Seakem

ME-agarose from FMC Bioproducts, Rockland, ME, U.S.A., nylon (Hybond-N) from Amersham Corp., and ^{32}P -labelled nucleotides from du Pont–New England Nuclear. Additional chemicals were purchased from Sigma (St. Louis, MO, U.S.A.) and E. Merck AG (Darmstadt, Germany). Sprague–Dawley rats were purchased from Harlan Sprague–Dawley (Indianapolis, IN, U.S.A.) and were maintained on normal diets.

RNA isolation

Sprague–Dawley rats were killed at different stages of development: day 18 of gestation, newborn, 2 days old and adult rats. To obtain the fetal rats, the animals were mated overnight. Pregnancy was determined by the presence of a vaginal plug, and the gestation age of fetuses was calculated on this basis. Lung and kidneys were removed, washed in phosphate-buffered saline (PBS; 137 mM-NaCl/2.7 mM-KCl/8 mM- Na_2HPO_4 /1.47 mM- KH_2PO_4 , pH 7.2) and frozen in liquid N_2 . A portion (1 g) of frozen tissue was ground to a powder, resuspended in guanidinium isothiocyanate, and the RNA was isolated by CsCl-density-gradient centrifugation (Chirgwin *et al.*, 1979).

RNA-blot analysis

RNA samples were denatured in formaldehyde-containing buffer and electrophoresed in 1% agarose/2.2 M-formaldehyde gels as described previously (Gitlin, 1988). Under these conditions, 28 S rRNA was shown to run to the 4.0 kb position. After electrophoresis the RNA was revealed by ethidium bromide staining to ensure that equivalent amounts of intact total RNA were present for each sample. The RNA was then transferred to nylon membranes by capillary blotting and immobilized by exposure to u.v. light.

Northern-blot hybridization

An oligonucleotide corresponding to bp 780–840 of published DPP IV cDNA sequence data (Ogata *et al.*, 1989) was labelled with [^{32}P]ATP using T4 polynucleotide kinase (Maniatis *et al.*, 1982). The sequence of the oligonucleotide is shown in Fig. 1(a). The RNA blots were wetted in $5\times$ SSPE [prepared from a stock

Abbreviations used: PBS, phosphate-buffered saline; DPP IV, dipeptidyl peptidase IV.

* This paper is dedicated to Professor Alfred Hildebrandt.

Fig. 1. Specification of oligonucleotides used in the present study

(a) Sequence of the oligonucleotide used to hybridize the Northern blots for the detection of complementary transcripts (see the Materials and methods section). The oligonucleotide sequence is complementary to bp 780–840 of the published DPP IV cDNA sequence (Ogata *et al.*, 1989). Upper line, bp 780–840 of published sequence data; lower line (underlined), oligonucleotide sequence. (b) Sequence of the oligonucleotide used to probe 28 S rRNA in dot-blot analysis (see the Materials and methods section). It should be noted that the sequence shown above differs from the oligonucleotide used in the reference (Barbu & Dautry, 1989) in base 7 ('C' in human; 'G' in rat).

solution of $20 \times$ SSPE, containing 3 M-NaCl, 0.25 M- NaH_2PO_4 , 1 mM-EDTA, pH 7.4, as described by Maniatis *et al.* (1982)] and prehybridized at 55 °C in a solution containing $5 \times$ Denhardt's solution, $5 \times$ SSPE, 0.1 % SDS, 0.05 % sodium pyrophosphate and yeast tRNA (100 $\mu\text{g}/\text{ml}$). Hybridization was performed in the same solution for 12 h at 55 °C in the presence of the ^{32}P -labelled oligonucleotide. After hybridization, the filters were washed for 15 min in $6 \times$ SSC [prepared from a stock solution of $20 \times$ SSC containing 3 M-NaCl and 0.25 M-sodium citrate, pH 7.0, as described by Maniatis *et al.* (1982)] at room temperature twice, then for 1 min at 48 °C and for 1 min at 55 °C, again in $6 \times$ SSC. The filters were then exposed to a Kodak X-Omat film at -80 °C with a du Pont intensifying screen for 24 and 44 h. The RNA blots were re-probed with a cDNA for rat β -actin as previously described (Fleming & Gitlin, 1990) to ensure that equivalent amounts of RNA were present in each lane. The autoradiographs were scanned using a video densitometer (Biotech, Rosenheim, Germany).

RNA dot-blot analysis

RNA dot blots were performed as described by White & Bancroft (1982). Yeast tRNA and vector pBR 322 were used as controls to assess background binding. All dot-blots were done in serial dilution from 0.5–4.0 μg of total RNA/sample, and after autoradiography the individual dots corresponding to the 4.0 μg values were excised and quantified by liquid-scintillation counting. At each time point the radioactivity in the corresponding 28 S rRNA was used to normalize the values obtained for the DPP IV mRNA. A 26-mer oligonucleotide corresponding to a region of rat 28 S RNA (Chan *et al.*, 1983) was synthesized and labelled as described above and used in 10-fold molar excess for hybridization to RNA blots (Barbu & Dautry, 1989). The sequence of the oligonucleotide is displayed in Fig. 1(b).

DPP IV protein analysis

Rat lung and kidneys were removed at the same stages of development as mentioned above. At each stage, the specific organs of five animals were pooled, washed in PBS, homogenized and solubilized in a buffer containing 25 mM-Tris/HCl (pH 7.4)/150 mM-NaCl/0.75 % Triton-X 100 and centrifuged at 10000 g for 1 h. DPP IV activity and protein concentration in the supernatants were determined.

DPP IV immunoprecipitation

Protein A-Sepharose (7 mg) was incubated with a monoclonal antibody raised against DPP IV (Hartel *et al.*, 1989) in 5 ml of binding buffer [150 mM-NaCl/10 mM-Tris/HCl (pH 8.0)/1 mM-EDTA/0.75 % Triton-X 100] overnight, pelleted and washed twice. The amount equivalent to 5 mg of total protein from each organ supernatant was added to, and incubated with, the Protein A-bound antibody at 4 °C overnight. After centrifugation and removal of the supernatants, the pellets were resuspended and washed with binding buffer six times, using a lower detergent concentration each time. The last wash was done with PBS, pH 8.0. After addition of sample buffer the samples were boiled and centrifuged. The supernatants were analysed by SDS/PAGE (Laemmli, 1970).

In addition to the organs mentioned above, solubilization supernatants were prepared from different adult rat organs: kidney, liver, lung, heart, skeletal muscle, brain and proximal small intestine. Microsomal fractions were prepared from these organs by homogenization in STKM buffer (0.25 M-sucrose/50 mM-Tris/HCl (pH 7.4)/25 mM-KCl/5 mM- MgCl_2), centrifugation at 3000 rev./min (1200 g , 10 min) to remove the nuclear fraction, and further centrifugation in a KONTRON TFT 28.38 rotor at 100000 g for 1 h. The supernatants were recovered.

Determination of DPP IV activity

DPP IV activity in the homogenates as well as in the microsomal fractions was measured with 0.2 mM-glycylproline *p*-nitroanilide tosylate (Bachem, Bubendorf, Switzerland) as described previously (Kreissel *et al.*, 1982).

Protein determination

Protein determination was carried out using the BCA protein assay reagent (Pierce, Rockford, IL, U.S.A.).

For comparison, the specific DPP IV activity in both the solubilization supernatants and the microsomal fractions was determined.

RESULTS AND DISCUSSION

The determination of the specific DPP IV activity in the tissue homogenates and microsomal fractions of various rat organs (see the Materials and methods section) shows that, in organs with a relatively high DPP IV activity, such as small intestine, liver, lung and kidney, the specific DPP IV activity in the microsomal fractions exceeds the activity in the homogenate fractions about 3-fold (Fig. 2). The highest enzymic activities

Fig. 2. Specific DPP IV activity in various rat organs

Specific DPP IV activity (munits/mg of protein) was determined in tissue homogenates (□) and microsomal fractions (■) of various rat organs (see the Materials and methods section). Abbreviation: PSI, proximal small intestine.

Fig. 3. Expression of DPP IV RNA in different organs

The Figure shows an autoradiograph of a Northern blot hybridized with a labelled oligonucleotide derived from the published DPP IV cDNA sequence (see the Materials and methods section); 20 μ g of total RNA from each organ shown was used per lane.

Fig. 4. Detection of DPP IV activity and immunoprecipitation of DPP IV from tissue homogenates at different stages of organ (kidney and lung) development

(a) Specific DPP IV activity in kidney (i) and lung (ii), with error bars indicating the range. (b) Immunoprecipitated DPP IV (110 kDa) by SDS/PAGE, together with the antibody heavy chain. The determinations of DPP IV activity were performed as double determinations in two different sets of animals. For details, see the Materials and methods section.

were detected in the kidney. No such activity was detected in the microsomal fraction of the brain.

The autoradiographs of the Northern blots hybridized with the DPP IV oligonucleotide (Fig. 3) displayed two distinct bands: one of 3.2 kb, and thus equivalent to the presumptive size of DPP IV mRNA (Hong & Doyle, 1987), and another band of approx. 4.8 kb. The sizes of the transcripts were confirmed by using radiolabelled RNA markers generated from cRNA templates of known size. Both bands are most prominent in kidney RNA, corresponding to the high DPP IV activity in the kidney. The bands in lung and liver RNA appear to be of similar intensity, although both organs show significant differences in specific DPP

Fig. 5. DPP IV RNA expression during different stages of organ development

The Figure shows an autoradiograph of a Northern blot hybridized with a labelled oligonucleotide derived from the published DPP IV sequence; 20 μ g of total RNA was used per lane.

Fig. 6. DPP IV mRNA dot-blot analysis

The results of dot-blot analysis are presented as c.p.m. of DPP IV mRNA radioactivity obtained as outlined in the Materials and methods section and after normalization at each point using the 28 S RNA radioactivity. The data represent values ($\pm$ s.d.) derived from dot blots done in duplicate on RNA samples from four different animals or pools of animals at each time point. Day 3-5 represents the data obtained from animals two of which were day three and the other two day five. It should be noted that the data account for both DPP IV mRNAs shown in Figure 6.

IV activity in that the activity in lung exceeds the activity in liver by about 3-fold. Bands could also be detected in the RNA isolated from heart, whereas no bands were seen in the preparations of skeletal-muscle and brain RNA.

Two different forms of DPP IV, a membrane-bound and a soluble form, were described previously (Fukasawa *et al.*, 1983), but were suggested to be derived from a single transcript (Ogata *et al.*, 1989). Therefore the nature of the extra transcript remains to be elucidated and requires further study.

Fig. 4(a) shows the specific DPP IV activity in kidney and lung during different stages of development. The results obtained in two different sets of animals were averaged, revealing a similar increase in DPP IV enzymic activity in lung and kidney during organ development.

The intensity of the bands obtained in the immunoprecipitations (Fig. 4b) corresponds to the enzymic activity in the homogenate fractions. We conclude, therefore, that the increase in DPP IV activity during the development of lung and kidney is paralleled by an increase in DPP IV protein.

It should be noted that autoradiographs of Northern blots with RNA isolated at different stages of lung and kidney development hint at organ-specific differences (Fig. 5). In kidney

RNA, an increasing intensity of the two distinct bands in the different stages of kidney development can be observed. By contrast, the intensity of the bands detected in the preparations of RNA isolated at different stages of lung development does not increase, and thus does not parallel the increase in DPP IV protein and enzymic activity in this organ.

Re-probing of all RNA blots with a β -actin cDNA and quantification by densitometry revealed that equivalent amounts of RNA were present in each lane (results not shown).

To quantify the changes in DPP IV-specific transcripts in lung and kidney during development, RNA samples similar to those shown in Fig. 5 were subjected to dot-blot analysis. Identical blots were hybridized to oligonucleotides specific for either DPP IV or 28 S RNA. As Fig. 6 shows, little or no change in lung specific DPP IV mRNA was observed throughout development. Under the conditions used the hybridization was linear with respect to input RNA, and the abundance of 28 S RNA was similar in each sample, allowing internal standardization (results not shown). The data support the conclusion that translational or post-translational mechanisms determine the increase in DPP IV activity and protein in the lung during development and corroborate previous observations regarding transcriptional regulation of DPP IV expression in the kidney (Hong *et al.*, 1989).

Discrepancies between the amount of protein synthesized and the related mRNA have been described for other proteins, such as the β -myosin heavy chain during muscle atrophy (Thomason *et al.*, 1989) and thymidine kinase in serum-stimulated cells (Ito & Conrad, 1990). Another report (Büller *et al.*, 1990) suggested, for the expression of lactase-phlorizin hydrolase mRNA and enzyme levels in rat intestine, both transcriptional and post-transcriptional factors of regulation, depending on the stage of development. Organ-specific mechanisms for the regulation of DPP IV expression within the same organism may provide a valuable model for further studies on the post-transcriptional factors influencing DPP IV expression, since both mechanisms can be compared within the same organism and at the same point of time.

Although considerable effort was undertaken to show the existence of an active inhibitor of DPP IV in rat lungs at early stages of development, no such inhibitory effect could be observed by co-incubation of homogenate fractions from adult kidney and fetal, newborn, 2-day-old and adult-rat lung (results not shown).

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