Identification of cell surface dipeptidylpeptidase IV in human fibroblasts

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An antigen with dipeptidylpeptidase IV activity was identified at the surface of normal human fibroblasts. Hydrophobic interaction electrophoresis in phenyl–Sephacrose revealed that the enzyme contained a hydrophobic domain, while lactoperoxidase-catalysed iodination with ^{125}I of living cells indicated that the protein was located at the cell surface. Crossed immunoelectrophoresis with specific antibodies of acid-extracted or papain-treated cells showed a shift of the dipeptidylpeptidase IV peak to a faster mobility. The molecular properties of the fibroblast enzyme were clearly different from those described for dipeptidylpeptidase IV from other tissues and species. Fibroblast dipeptidylpeptidase IV contained two different disulphide-linked subunits, of apparent M_r values 125 000 and 135 000 (denatured and reduced). In gel filtration, an M_r of about 400 000 was observed for the unreduced molecule. The enzymic properties of fibroblast dipeptidylpeptidase IV were very similar to those of the well-characterized pig kidney enzyme. Activity towards glycyl-L-prolyl- β -naphthylamide was inhibited 50% by 0.023 mM-di-isopropylphosphorofluoridate. L-Alanyl-L-alanyl- β -naphthylamide was hydrolysed ten times more slowly than glycyl-L-prolyl- β -naphthylamide.

Recently, Verlinden *et al.* (1981a) described the application of crossed immunoelectrophoresis to the characterization of cellular antigens of cultured fibroblasts. Membrane-bound aminopeptidase M was identified in the crossed immunoelectrophoresis pattern of normal human fibroblasts, with a poly-specific anti-(human fibroblast) serum (Verlinden *et al.*, 1981b,c). Characteristically, this antigen migrated faster in crossed immunoelectrophoresis when cells had been extracted at pH 5.5. When the effect of acid treatment was further examined, another antigen which behaved similarly to aminopeptidase was detected.

Here we present the characteristics of this antigen. The immunoprecipitate stained enzymically in the presence of Gly-L-Pro- β -NNap, a substrate specific for dipeptidylpeptidase IV (EC 3.4.14.5) (Hopshu-Havu & Sarimo, 1967). When the structural and enzymic properties of this fibroblast dipeptidylpeptidase IV were examined and compared with those described for enzymes from other tissues, important differences were noted in molecular weight and subunit composition.

Abbreviations used: β -NNap, β -naphthylamide; SDS, sodium dodecyl sulphate; IgG, immunoglobulin G.

Experimental

Materials

Phenyl–Sephacrose CL-4B was purchased from Pharmacia. Berol EMU-043 was from Berol Kemi AB, Sweden. Gly-L-Pro- β -NNap, L-Ala-L-Ala- β -NNap and L-Ala- β -NNap came from Bachem Feinchemikalien AG, Bubendorf, Switzerland. Papain was from Sigma. Pansorbin beads were purchased from Calbiochem-Behring, and di-isopropylphosphorofluoridate from Aldrich. Peroxidase-linked swine anti-(rabbit IgG) sera were from Dakopatts, Copenhagen, Denmark. [^{35}S]Methionine (1240 Ci/mmol), [^{14}C]fucose (250 mCi/mmol) and Na ^{125}I (100 mCi/ml) were from The Radiochemical Centre, Amersham.

Bestatin was a gift from Dr. H. Umezawa, Institute of Microbial Chemistry, Microbial Chemistry Research Foundations, Tokyo, Japan.

Crossed immunoelectrophoresis

Crossed immunoelectrophoresis was done as described (Verlinden *et al.*, 1981a). Hydrophobic-interaction crossed immunoelectrophoresis was performed according to the procedure of Bjerrum (1978), in which phenyl–Sephacrose CL-4B beads

were incorporated in the first-dimension agarose gel. Berol (1%) was present in the second-dimension gel.

The immunoprecipitates were stained with Coomassie Brilliant Blue R-250 as described by Verlinden *et al.* (1981a). Enzymic staining of immunoprecipitates for dipeptidylpeptidase IV (or aminopeptidase M) activity was performed on wet gels by coupling β -naphthylamide, the product from splitting Gly-Pro- β -N^{ap} (or Ala- β -N^{ap}), to Fast Blue B salt according to Nachlas *et al.* (1957). Enzymic staining for maltase, isomaltase and sucrase, performed on crossed immunoelectrophoresis plates, was as described by Danielsen *et al.* (1977). The procedure for neutral endopeptidase activity staining on crossed immunoelectrophoresis plates was from Booth *et al.* (1979). Autoradiography was done as described by Verlinden *et al.* (1981a).

Preparation of cells for crossed immunoelectrophoresis

Cell culture and harvesting of human diploid fibroblasts (NHF) by trypsinization was done as described by Verlinden *et al.* (1981a). The resulting cell pellets were stored at -20°C . Extracts were prepared by one of the following procedures.

(a) *Detergent extraction.* The frozen cell pellets were thawed, resuspended in 4 vol of 75 mM-Veronal buffer (pH 8.6) containing 2% Berol (room temperature) and sonicated immediately. Insoluble material was removed by centrifugation in a Beckman Airfuge at 130 000 g (10^5 rev./min) for 6 min.

(b) *Acid-detergent extraction.* Thawed cell pellets were resuspended in 4 vol of 0.16 M-sodium acetate buffer (pH 5.5) containing 2% Berol. The suspension was sonicated and incubated for 60 min at 37°C . Insoluble material was removed by centrifugation as described above.

(c) *Papain extraction.* Papain (0.01% and 0.1%) was activated in phosphate-buffered saline (137 mM-NaCl, 8.1 mM- Na_2HPO_4 , 1.5 mM- KH_2PO_4 , 2.7 mM-KCl, 15 mM- NaN_3) containing 0.02% EDTA and 5 mM-cysteine, by incubation at 37°C for 30 min. The thawed cell pellets were resuspended in 4 vol. of phosphate-buffered saline and sonicated. This preparation was centrifuged (130 000 g for 6 min), and the pellet washed once with phosphate-buffered saline. This pellet was resuspended in the activated papain solution, in the presence or absence of 2% Berol as specified, and incubated for 30 min at 37°C . After addition of 270 mM-iodoacetamide, insoluble material was removed by centrifugation as described above.

Radioactive labelling of cells

Cellular antigens were labelled either metabolically by incorporation of [^{35}S]methionine and [^{14}C]fucose, or exogenously by enzymic iodination. [^{35}S]Methionine was added at 8 $\mu\text{Ci/ml}$ to methion-

ine-free Dulbecco's modified Eagle's medium (Flow Laboratories) containing 10% (v/v) newborn calf serum. After 24 h incubation in this medium, the cells were washed in phosphate-buffered saline, collected and treated as above.

Metabolic carbohydrate labelling was carried out by incubation of the cell layers with serum-free medium supplemented with [^{14}C]fucose (1.2 $\mu\text{Ci/ml}$) for 6 h.

Lactoperoxidase-catalysed iodination with Na^{125}I was carried out as described (Hynes, 1973).

Preparation of antisera

Anti-fibroblast serum (aNHfiv) was obtained by intravenous injection of rabbits with whole cells as described (Verlinden *et al.*, 1981b).

Antibodies directed against dipeptidylpeptidase IV were obtained as follows. Immunoprecipitates of the acid-extracted form of dipeptidylpeptidase IV were excised from an unstained crossed immunoelectrophoresis agarose gel under dark-field illumination and suspended in 3 vol. of water by sonication. Immunoprecipitates, equivalent to 5×10^6 cells, were mixed with 1 ml of complete Freund's adjuvant and injected intradermally into rabbits at 2 week intervals. After three injections the rabbits were bled. Hyperimmune IgG was isolated as described by Verlinden *et al.* (1981a).

Polyacrylamide-gel electrophoresis

SDS/polyacrylamide-gel electrophoresis was performed as described by Laemmli (1970) on gradient slab gels (6–20% and 4–15% polyacrylamide). Immunoprecipitates, excised from a wet crossed immunoelectrophoresis gel as described above, were dissolved in sample buffer containing 2% SDS and 5% 2-mercaptoethanol by boiling for 5 min.

Protein bands were electrophoretically transferred from polyacrylamide gels to nitrocellulose filters (Millipore) as described (Towbin *et al.*, 1979). The filters were inactivated overnight in phosphate-buffered saline containing 0.5% casein at 37°C . Incubation with specific antibodies (diluted 1:20 in phosphate-buffered saline/casein) was for 1 h at room temperature. The filter paper was subsequently washed three times in phosphate-buffered saline/casein (10 min each) and treated with peroxidase-linked swine anti-(rabbit IgG) (1:50 dilution). After 1 h at room temperature the filters were washed three times in phosphate-buffered saline/casein and once in 50 mM-Tris/HCl, pH 7.6. The staining reaction was done with 5 mg of diaminobenzidine and 3.3 μl of H_2O_2 (30%) in 10 ml of 50 mM-Tris/HCl (pH 7.6).

Gel filtration

High performance gel filtration was performed on a TSK 3000 column (LKB, Bromma, Sweden)

equilibrated with phosphate-buffered saline (pH 6.8). Preparation of the samples and other details are given in the legend to Fig. 5.

Solubilization of dipeptidylpeptidase IV from crossed immunoelectrophoresis immunoprecipitates

Immunoprecipitates of dipeptidylpeptidase IV were excised from the wet gel under dark-field illumination, suspended by sonication in 2 ml of 8 M-urea and left for 2 h at room temperature. Full enzymic activity was recovered after this treatment. Pansorbin beads (100 μ l of a 10% suspension) were added and the solution was dialysed with continuous stirring against 2 mM-Tris/HCl (pH 7.0) containing 0.1% Berol. After centrifugation at 10000 *g* for 10 min, 90% of the dipeptidylpeptidase IV activity was recovered in the supernatant, while 10% remained associated with the beads.

Enzymic activity

Samples of the solubilized antigens (see above) were made 50 mM in Tris/HCl (pH 7.8), containing 50 μ M-bestatin. After 2 h at 37°C, the solution was made 1 mM in Gly-Pro- β -NNap (or Ala-Ala- β -NNap) (final vol. 300 μ l) and dipeptidylpeptidase IV activity was determined spectrophotometrically by measuring the rate of liberation of β -naphthylamine from the amino acid derivatives at 340 nm. In these conditions the rate of substrate hydrolysis increased linearly as a function of time and enzyme concentration. Absorbance values were corrected for turbidity and spontaneous substrate hydrolysis. Enzyme activity is expressed as nmol of substrate hydrolysed/min using the absorption coefficient 1780 M⁻¹·cm⁻¹.

Results

Visualization of dipeptidylpeptidase IV in crossed immunoelectrophoresis

Crossed immunoelectrophoresis of normal human fibroblasts against an oligospecific anti-(human fibroblast) serum (aNHfiv), obtained by intravenous injection of rabbits with whole cells, revealed one major double precipitate, identified previously as aminopeptidase M (Verlinden *et al.*, 1981c). When cell extracts were incubated at pH 5.5 the slow migrating peak of aminopeptidase was converted into the fast-migrating precipitate. In addition, another sharp peak of slower mobility than, and intersecting with, the aminopeptidase precipitate became visible (Fig. 1b). Alkalinization of the acid extract (to pH 8.6) did not restore the pattern observed with pH 8.6 cell extracts, suggesting that acidification irreversibly altered the antigen. Specific antibodies against this newly visualized antigen were obtained by injecting rabbits with the immunoprecipitate, excised from unstained crossed immunoelectrophoresis plates. Crossed immunoelectrophor-

Fig. 1. *Detection and identification of dipeptidylpeptidase in crossed immunoelectrophoresis*

Crossed immunoelectrophoresis of human fibroblasts (approx. 4×10^5 cells), (a, c, e) extracted in 2% Berol, pH 8.6, or (b, d) acid-extracted at pH 5.5 (1 h at 37°C) against (a, b) the oligospecific anti-fibroblast serum (aNHfiv) or (c, d, e) specific antibodies against dipeptidylpeptidase IV. Gels (a) to (d) were stained with Coomassie Blue and (e) was stained enzymically with Gly-Pro- β -NNap (see the Experimental section). The aminopeptidase (AP) and the dipeptidylpeptidase IV (DPPIV) peaks are indicated in (a) and (b). The extreme left of the application well is indicated by an arrow.

esis of an alkaline cell extract (pH 8.6) with this antiserum resulted in a broad asymmetric precipitate, with β -mobility (Fig. 1c). In an acid cell extract (pH 5.5) the peak was sharper and of α -mobility (Fig. 1d). Increased mobility in crossed immunoelectrophoresis after acid extraction, probably due to endogenous proteolysis, was described for aminopeptidase M (Verlinden *et al.*, 1981c). Also, membrane hydrolases of kidney and intestine were shown to have an increased mobility in crossed immunoelectrophoresis after proteinase, compared with detergent extraction (Kenny & Maroux, 1982). Identification of the immunoprecipitates through enzymic staining with different substrates specific for brush border hydrolases was therefore attempted. Isomaltase, sucrase, maltase, aminopeptidase or neutral endopeptidase activity could not be demonstrated in the immunoprecipitates. Gly-Pro- β -NNap,

Table 1. *Dipeptidylpeptidase IV* activity in cell extracts

The equivalent of 1.6×10^5 intact trypsinized NHF fibroblasts, the supernatant of the same amount of sonicated cells centrifuged at $130\,000g$ for 6 min and the corresponding pellet solubilized in 2% Berol were tested for enzyme activity towards Gly-Pro- β -NNap. The samples were diluted in 50 mM-Tris/HCl (pH 7.8), containing 1 mM-Gly-Pro- β -NNap, and the change in A_{340} was measured spectrophotometrically at 37°C . Protein concentration was determined according to Lowry *et al.* (1951). The mean ($\pm$ S.E.M.) of four determinations is given.

Extract	Total activity (nmol/min)	Specific activity (nmol/min per mg of protein)
Intact cells	420 ± 34	562 ± 28
Supernatant	96 ± 11	225 ± 39
Pellet	348 ± 34	1388 ± 180

a substrate specific for dipeptidylpeptidase IV, however gave a clear staining of the peak (Figs. 1e and 2). In crossed immunoelectrophoresis against a polyspecific anti-fibroblast serum (aNHf) generating more than 20 visible peaks (Verlinden *et al.*, 1981a) only the peak under investigation stained enzymically with Gly-Pro- β -NNap (results not shown).

Membrane localization of dipeptidylpeptidase IV

Dipeptidylpeptidase IV was labelled metabolically by [^{35}S]methionine and [^{14}C]fucose (results not shown), indicating that it was a cellular glycoprotein. Moreover, the antigen was labelled by exogenous lactoperoxidase-catalysed ^{125}I iodination of living cells, suggesting its exposure at the cell surface (results not shown). Quantification of the enzyme activity in intact cells and in cell extracts indicated that the $130\,000g$ pellet of fibroblasts, sonicated in the absence of detergent, contained 78% of the recovered activity (Table 1). The enzyme activity could only be solubilized from the pellets by detergent extraction. This was confirmed in crossed immunoelectrophoresis, since the peak corresponding to dipeptidylpeptidase IV was only observed after incubation of the $130\,000g$ pellets of sonicated cells with 2% Berol. In addition, the dipeptidylpeptidase IV peak with a mobility obtained by acid-detergent extraction did not appear when detergent was omitted, even after 18 h incubation at pH 5.5 and 37°C . Treatment of cell extracts with papain gave comparable results. Fig. 2 illustrates the gradual shift in mobility from β to α of detergent-solubilized dipeptidylpeptidase IV exposed to increasing papain concentrations. A similar papain treatment in the absence of detergent did not solubilize dipeptidylpeptidase IV and subsequent addition of 2% Berol to the resuspended cell pellets

Fig. 2. Effect of papain digestion on the mobility of dipeptidylpeptidase IV

Fibroblasts were sonicated in the absence of Berol and centrifuged at $130\,000g$. The pellets were incubated for 30 min at 37°C in (a) phosphate-buffered saline containing 2% Berol; (b) 0.01% papain and 2% Berol in phosphate-buffered saline; (c) 0.1% papain and 2% Berol in phosphate-buffered saline. The samples were run in crossed immunoelectrophoresis against anti-(dipeptidylpeptidase IV). The first-dimension gel strips were cut at the extreme left of the application well, indicated by an arrow at the base of the Figure. The three upper arrows indicate a position, 1 cm to the left of the application well, coinciding with the top of the slow-migrating dipeptidylpeptidase IV peak. The peaks were visualized by staining with Gly-Pro- β -NNap.

yielded a peak which still migrated at β mobility in crossed immunoelectrophoresis (results not shown).

Finally, hydrophobic-interaction crossed immunoelectrophoresis was used to examine whether the antigen contained a hydrophobic domain. Phenyl-Sepharose beads were incorporated in the first-dimension gel of crossed immunoelectrophoresis (Bjerrum, 1978). Detergent-solubilized dipeptidylpeptidase IV was completely retained (Fig. 3a). Even during the second dimension electrophoresis in the presence of detergent, the dipeptidylpeptidase IV molecules were only partially detached from the hydrophobic beads. The acid-extracted α -peak, however, was minimally retarded (Fig. 3b).

M_r determinations

Immunoprecipitates of the acid-extracted α - and the detergent-solubilized β -forms of dipeptidylpeptidase IV, from ^{125}I /lactoperoxidase-labelled fibroblasts, were excised from unstained crossed immunoelectrophoresis gels and applied to parallel slots of a 6–20% polyacrylamide gradient slab gel (Fig. 4, lanes a and b). The autoradiograms showed that both forms of dipeptidylpeptidase IV consist of different subunits with apparent M_r values of 125 000 and 135 000. The large subunit of the α -form consistently showed a slightly higher mobility

Fig. 3. Hydrophobic interaction crossed immunoelectrophoresis

The detergent-solubilized 130 000 g pellet of NHF fibroblasts, which had been sonicated in the absence of Berol (approx. 10^6 cells) (a) and fibroblasts extracted in 2% Berol at pH 5.5 for 2 h at 37°C (b) were run against anti-(dipeptidylpeptidase IV). Phenyl-Sepharose beads were included in the first-dimension gel.

(lane b) than did the corresponding subunit of the β -form (lane a). Immunostaining on nitrocellulose replicas of the 6–20% gradient polyacrylamide gel (see the Experimental section) also revealed the two bands (Fig. 4, lane c). When treatment with 2-mercaptoethanol was omitted, however, the enzyme barely entered the gel (Fig. 4, lane d). In a 4–15% polyacrylamide gradient gel, unreduced dipeptidylpeptidase IV migrated somewhat slower than α_2 -macroglobulin half-molecules (M_r 360 000) used as marker (results not shown).

The native M_r of the enzyme was determined by high performance gel filtration of an acid cell extract containing dipeptidylpeptidase IV in the α -form and of a detergent extract which yields a dipeptidylpeptidase IV peak at the β position in crossed immunoelectrophoresis. Elution of dipeptidylpeptidase IV, monitored by measuring enzyme activity, corresponded to an M_r of 400 000 in both preparations (Fig. 5). An additional peak of activity in the high-molecular-weight range was observed for the detergent-extracted preparation.

Enzyme activity of the different forms of dipeptidylpeptidase IV

To determine the enzyme activity of dipeptidylpeptidase IV with different mobilities in crossed immunoelectrophoresis, the immunoprecipitates were excised from crossed immunoelectrophoresis agarose gels and dissociated in 8 M-urea (2 h at room temperature). Kidney dipeptidylpeptidase IV was shown to be stable under these conditions for at least

Fig. 4. SDS/polyacrylamide-gel electrophoresis

(a) and (b) are autoradiograms of 6–20% gradient slab gels of 125 I-labelled peaks of dipeptidylpeptidase IV excised from wet crossed immunoelectrophoresis plates. In (a) the detergent-solubilized form of dipeptidylpeptidase IV was applied; (b) contains the acid-extracted form. Lanes (c) and (d) were transferred to nitrocellulose by blotting; the detergent-solubilized form of dipeptidylpeptidase IV, excised from crossed immunoelectrophoresis plates, was applied, respectively treated with (c) and without (d) 5% 2-mercaptoethanol. Cold-insoluble globulin (M_r 220 000), β -galactosidase (M_r 135 000 and 67 000), RNA polymerase (M_r 165 000, 155 000 and 39 000) and ovalbumin (M_r 45 000) were used as markers.

4 h (Barth *et al.*, 1974) and similarly we found no decrease in enzyme activity on fibroblast dipeptidylpeptidase IV by this treatment. Svensson *et al.* (1978) observed that intestinal dipeptidylpeptidase IV was eluted from immobilized specific antibodies with 2 mM-Tris/HCl buffer (pH 7). We therefore dialysed the 8 M-urea preparations against this buffer in order to dissociate the immune complexes. An excess of Pansorbin beads, added to remove IgG and any remaining antigen–antibody complexes, bound only 10% of the total dipeptidylpeptidase IV activity. In SDS/polyacrylamide-gel electrophoresis the two characteristic bands were observed for both forms of the solubilized antigen. The hydrolysis of two substrates, Gly-Pro- β -NNAp and Ala-Ala- β -NNAp, specific for kidney dipeptidylpeptidase IV, was measured. Since the presence of small amounts

Fig. 5. High-performance gel filtration of dipeptidylpeptidase IV

Two different cellular preparations were analysed on a TSK 3000 gel filtration column (LKB). ●, detergent-extracted fibroblasts (the equivalent of 6×10^6 cells); ○, acid-detergent extracted cells (the equivalent of 4×10^6 cells). The column was eluted at 2 ml/h and 0.5 ml fractions were collected. Aliquots (100 μ l) were analysed for dipeptidylpeptidase IV content by diluting in 2 ml of 50 mM-Tris/HCl (pH 7.8), containing 1 mM-Gly-Pro- β -NNap, and incubation at 37°C. Arrows indicate the elution position of markers: α_2 macroglobulin (α_2 M, M_r 725 000); fibronectin (FbN, M_r 440 000) and aminopeptidase M (AP, M_r 340 000).

of aminopeptidase would influence the measurements, both by stepwise cleavage of Ala-Ala- β -NNap (Barth *et al.*, 1974) or by altering the kinetics of dipeptidylpeptidase IV activity (Wolf *et al.*, 1978), the specific aminopeptidase inhibitor bestatin (Umezawa *et al.*, 1976) was added to the test medium. In agreement with Hazato *et al.* (1982) we found that 50 μ M-bestatin had no influence on dipeptidylpeptidase IV activity. Samples of both the acid-extracted α form and the alkaline-solubilized β form of dipeptidylpeptidase IV hydrolysed Ala-Ala- β -NNap 10 times more slowly than Gly-Pro- β -NNap. The sensitivity of the Gly-Pro- β -NNap hydrolysing activity of dipeptidylpeptidase IV to di-isopropylphosphorofluoridate was also measured. Fractions of the acid extracted α form of dipeptidylpeptidase IV were preincubated for 90 min with different di-isopropylphosphorofluoridate concentrations in 50 mM-Tris/HCl (pH 7.8) at 37°C. Then Gly-Pro- β -NNap was added to a final concentration of 1 mM. The rate of hydrolysis of the control sample was 187 nmol/min; 50% inhibition was obtained with 0.023 mM-di-isopropylphosphorofluoridate.

Discussion

Hopsu-Havu & Ekfors (1969) have shown that dermal fibroblasts, especially those located around hair follicles, display high dipeptidylpeptidase IV activity, while human skin represented only a minor source of dipeptidylpeptidase IV activity compared with other tissues (Hopsu-Havu & Ekfors, 1969; De Bersaques, 1970). We identified dipeptidylpeptidase IV in human fibroblasts by direct enzymic staining of the crossed immunoelectrophoresis gels with Gly-Pro- β -NNap. The solubilized antigen had similar enzymic properties to the well-characterized pig kidney dipeptidylpeptidase IV (Barth *et al.*, 1974; Kenny *et al.*, 1976). The molecule was membrane-associated, located at the outer cell-surface and it contained a hydrophobic domain. In the presence of detergent the hydrophobic domain was lost upon acid treatment at pH 5.5, also resulting in an increased mobility in crossed immunoelectrophoresis. This was probably due to the action of lysosomal enzymes. Papain treatment had a similar effect. Macnair & Kenny (1979) and Booth & Kenny (1980) described an M_r 4000 hydrophobic anchor for kidney dipeptidylpeptidase IV. The fibroblast dipeptidylpeptidase IV hydrophobic domain must also be small since no major difference in M_r value was observed between acid- and detergent-solubilized molecules in gel filtration. In SDS/polyacrylamide gel electrophoresis after reduction, only the subunit of M_r 135 000 appeared to migrate somewhat faster.

Dipeptidylpeptidase has been isolated from different tissues such as rat liver (Hopsu-Havu & Sarino, 1976), human submaxillary gland (Oya *et al.*, 1972); pig kidney (Barth *et al.*, 1974; Kenny *et al.*, 1976), lamb kidney (Yoshimoto & Walter, 1977), human kidney (Hama *et al.*, 1982), pig intestine (Svensson *et al.*, 1978), pig pancreas (Yoshimoto *et al.*, 1982) and human placenta (Püschel *et al.*, 1982). All these molecules displayed a native M_r between 220 000 and 280 000. It was therefore surprising to find that human fibroblast dipeptidylpeptidase IV had an M_r of 400 000 in gel filtration. In addition, the enzyme is apparently composed of different subunits (M_r 125 000 and M_r 135 000) linked by disulphide bonds. This again contrasts with the enzymes previously described (see above) since these were dissociated, by denaturation only, into two subunits with an identical M_r in the range of 110 000–137 000. The high-molecular-weight peak seen in gel filtration of the detergent-solubilized β form of dipeptidylpeptidase IV is probably due to formation of lipid-detergent micelles.

It is interesting to note that an M_r 400 000 peak, with dipeptidylpeptidase IV activity, was seen in gel filtration of human urine (Kato *et al.*, 1978). Preliminary results indicate that this molecule is at

least immunologically related to fibroblast dipeptidylpeptidase IV, since antibodies directed against human urine proteins precipitate dipeptidylpeptidase IV from fibroblast cell extracts in crossed immunoelectrophoresis (M. Saison, J. Verlinden, F. Van Leuven, J.-J. Cassiman & H. Van den Berghe, unpublished results).

In conclusion, our investigations demonstrated the presence of dipeptidylpeptidase IV activity at the cell membrane of human fibroblasts. Together with aminopeptidase M (Verlinden *et al.*, 1981c), it is the second *N*-terminal peptidase present in large amounts. Whether the occurrence of these enzymes is important for a particular activity of fibroblasts remains to be determined.

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