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Immunological cross-reactivity between human tripeptidyl peptidase II and fibronectin

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Tripeptidyl peptidase II (TPP II) is a large intracellular exopeptidase with an active site of the subtilisin type. Affinity-purified hen antibodies against human erythrocyte TPP II cross-reacted with fibronectin in an immunoblot analysis. Furthermore, antibodies against human fibronectin cross-reacted with TPP II. Antibodies against a 65 kDa cell-binding fragment of fibronectin specifically reacted with TPP II, whereas antibodies against the collagen-binding domain, the main heparin-binding domain or the *N*-terminal fibrin-binding domain did not react. Moreover, the affinity-purified antibodies against TPP II reacted with a 105 kDa cell-binding fragment of fibronectin but not with the fibrin-binding domain or the collagen-binding domain. When native TPP II was dissociated into smaller units through dialysis against a dilute Tris buffer, it could be digested by chymotrypsin into three stable fragments of 70 kDa, 42 kDa and 20 kDa. It could be demonstrated that the 42 kDa fragment was specifically recognized by antibodies against the 65 kDa cell-binding fragment of fibronectin. Furthermore, labelling with di-[^{3}H]isopropyl phosphorofluoridate and *N*-terminal sequence determination showed that the 70 kDa fragment contained the active-site serine residue. In conclusion, our findings suggest that one domain of the TPP II molecule bears structural resemblance to a cell-binding fragment of fibronectin.

INTRODUCTION

Tripeptidyl peptidase II (TPP II) is an exopeptidase [1] of an unusually large size, i.e. more than 1000 kDa for the native enzyme and 135 kDa for the subunit [1,2]. Upon electron microscopy of negatively stained samples [3], the enzyme displays a few ordered polymeric structures; these can be dissociated into smaller units upon dialysis against a dilute Tris buffer. The dissociation is accompanied by a gradual loss of enzymic activity, indicating that the large polymeric form is a prerequisite for full enzymic activity. The enzyme, which has a neutral pH optimum, has been classified as a serine peptidase [2]. The amino acid sequence around the active-site serine residue is of the subtilisin type [4]. This is in contrast with the other known mammalian serine peptidases, which have an active site of the trypsin type [5].

TPP II was originally detected in rat liver [1], but is present in several other rat tissues [2]. The enzyme has also been shown to be present in haemolysates and liver homogenates from other species [6], as seen both by activity measurements and by the use of antibodies raised against the human erythrocyte enzyme. During attempts to determine the subcellular location of TPP II in human fibroblasts by immunohistochemical techniques, it was noticed that the affinity-purified polyclonal hen antibodies against TPP II reacted towards material with a distribution similar to that of fibronectin (R. Persson, B. Tomkinson & Ö. Zetterqvist, unpublished work). In the present work we have investigated the immunological cross-reactivity between TPP II, an intracellular enzyme, and fibronectin, an extracellular matrix protein involved in cell–matrix interactions [7], with particular reference to the domain structures of the two proteins.

EXPERIMENTAL

Materials

Di-[1,3-^{3}H]isopropyl phosphorofluoridate and Amplify were purchased from Amersham International (Amersham, Bucks., U.K.). Calibration proteins for gel electrophoresis, CNBr-activated Sepharose 4B, AH-Sepharose 4B and prepacked Sephadex G-25 columns were procured from Pharmacia (Uppsala, Sweden). BSA (fraction V), chymotrypsin (type II), Nitro Blue Tetrazolium and 5-bromo-4-chloroindol-3-yl phosphate were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Immobilon [poly(vinylidene difluoride)] membranes came from Millipore (Bedford, MA, U.S.A.) and nitrocellulose filters (0.2 μm pore size) from Schleicher und Schüll (Dassel, Germany). Glycerol was bought from Dow Chemicals (Detroit, MI, U.S.A.) and met U.S.P. requirements, and the poly(ethylene glycol) 6000 (puriss grade) was obtained from Kebo Lab A.B. (Spånga, Sweden). Human foreskin fibroblasts (AG 1523) were grown by Charlotte Rorsman at the Ludwig Institute for Cancer Research, Uppsala, Sweden.

Human plasma fibronectin, purified as described by Vuento & Vaheri [8], and fragments thereof were kindly donated by Dr. Staffan Johansson of this Department. TPP II was prepared from outdated human erythrocytes, essentially as described previously [2,4]. The resolution in the hydroxyapatite chromatography, used as the last purification step, was improved by stepwise elution with 3 column volumes each of 10 mM-, 50 mM- and 60 mM-potassium phosphate buffer, pH 7.5, containing 30% (w/v) glycerol and 1 mM-dithiothreitol. The enzyme was eluted at the 50 mM-phosphate concentration.

Hen antibodies against human TPP II were obtained from Immunsystem A.B. (Uppsala, Sweden). A portion (2 ml containing 27 mg of protein) of the antibody solution was affinity-purified on an AH-Sepharose column conjugated with the denatured 135 kDa peptidase subunit [6]. The eluates from two runs were desalted on Sephadex G-25, pooled, reloaded on to the affinity column and processed through a second chromatography cycle. These affinity-purified antibodies (0.29 mg of protein/ml) were used throughout the work. Rabbit antiserum against human plasma fibronectin and affinity-purified hen antibodies against human plasma fibronectin fragments [9] were generously donated by Dr. Staffan Johansson. Hen antibodies against human plasma fibronectin and alkaline phosphatase-conjugated rabbit anti-

Abbreviation used: TPP II, tripeptidyl peptidase II.
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(hen IgG) antibodies came from Immunsystem A.B., and alkaline phosphatase-conjugated pig anti-(rabbit IgG) antibodies came from Dakopatts (Glostrup, Denmark).

Electrophoresis

PAGE was performed in the discontinuous SDS/PAGE system of Laemmli as described by O'Farrell [10]. The acrylamide concentration of the running gel was 8% or 11% (w/v), as indicated. Before application, sample buffer was added to the samples, giving final concentrations of 2.3% (w/v) SDS, 5% (v/v) 2-mercaptoethanol and 10% (w/v) glycerol. The mixtures were heated in a boiling-water bath for 3 min. After electrophoresis, the gel was either treated for 1 h with 10% (w/v) trichloroacetic acid, stained with Coomassie Brilliant Blue and destained [11], or fixed and silver-stained [12].

Immunoblot analysis

SDS/polyacrylamide gels, run as described above, and nitrocellulose filters were equilibrated in transfer buffer [20 mM-Tris/150 mM-glycine buffer, pH 8.4, containing 10% (v/v) methanol and 0.01% SDS] for 20 min at room temperature. The polypeptides were then transferred electrophoretically from the gel to the nitrocellulose filter in a Trans-Blot cell (Bio-Rad Laboratories, Richmond, CA, U.S.A.) for 6 h at 10 V/cm in the transfer buffer. By this procedure, a complete transfer of the smaller fragments was obtained, whereas less than 10% of the TPP II and about 25% of the fibronectin remained in the gel, as judged from Coomassie Blue-staining of the gel after transfer. After transfer, the nitrocellulose filter was incubated overnight at 4 °C with 3% (w/v) gelatine in phosphate-buffered saline (20 mM-sodium phosphate buffer, pH 7.5, containing 150 mM-NaCl) and thereafter for 2 h at room temperature with antibodies diluted in phosphate-buffered saline containing 1% (w/v) BSA and 3% (w/v) poly(ethylene glycol). The filtered were washed for 30 min with three changes of washing buffer [0.5% BSA, 0.1% Tween-20 and 3% poly(ethylene glycol) in phosphate-buffered saline]. Alkaline phosphatase-conjugated secondary antibodies, diluted according to the manufacturer's recommendations, in the same buffer as used for primary antibodies, were added and the filters were incubated for an additional 2 h at room temperature. The filters were washed for 40 min with the washing buffer and the antigen was detected by incubation for 20 min–2 h with Nitro Blue Tetrazolium (0.01%) and 5-bromo-4-chloroindol-3-yl phosphate (0.05 mg/ml) in 0.1 M-ethanolamine/HCl buffer, pH 9.6, containing 3.6 mM-$MgCl_2$. As a control, all immunoblot experiments were also performed with pre-immune antibodies, whereby no detectable staining was seen (results not shown).

Fibronectin–Sepharose column

Fibronectin (5 mg) was dialysed overnight against 0.1 M-sodium bicarbonate buffer, pH 8.3, containing 0.5 M-NaCl, and thereafter linked to 0.3 g of CNBr-activated Sepharose 4B according to the manufacturer's recommendations. The gel (1 ml) was equilibrated in phosphate-buffered saline at pH 7.0 containing 0.1% Tween-80. Affinity-purified hen antibodies against TPP II (2 ml, containing 0.6 mg of protein), prepared as described above, were loaded on to the fibronectin–Sepharose column, and the affinity column was washed and eluted with $MgCl_2$ in the same way as previously described for the TPP II–Sepharose column [6]. The fractions were analysed by immunoblot analysis as described in Fig. 2.

Dissociation of TPP II

TPP II [in 50 mM-potassium phosphate buffer, pH 7.5, containing 30% (w/v) glycerol and 1 mM-dithiothreitol] was dialysed for 4 h at 4 °C against 2 mM-Tris/HCl buffer, pH 8.0, containing 3% (w/v) glycerol and 0.5 mM-2-mercaptoethanol, as described previously [3].

Cell-binding assay

Native TPP II [diluted in 50 mM-potassium phosphate buffer, pH 7.5, containing 30% (w/v) glycerol and 1 mM-dithiothreitol], dissociated TPP II (diluted in the dialysis buffer), chymotrypsin-digested TPP II (prepared as described in the legend to Fig. 7) and human fibronectin (diluted in 137 mM-NaCl/4.7 mM-KCl/0.6 mM-$MgSO_4$/1.2 mM-$CaCl_2$/10 mM-Hepes/NaOH buffer, pH 7.4, i.e. buffer 3 [13]) were all diluted to 2.5 and 12.5 μg/ml, and 0.4 ml of each solution was added to wells in a 24-well plate (Costar). The protein was allowed to adsorb for 1.5 h at 37 °C. Remaining protein-binding sites were blocked by using 0.25 mg of BSA/ml in buffer 3. After 20 min at 37 °C, the wells were washed, once with buffer 3 and once with Dulbecco's modified Eagle's medium (GIBCO, Grand Island, NY, U.S.A.) [14,15]. Hepatocytes, 4×10^5 in 0.4 ml of Dulbecco's modified Eagle's medium, isolated from male Sprague–Dawley rats after perfusion of the liver with collagenase as described by Rubin *et al.* [13], were seeded in each well and incubated at 37 °C. After 1 h, the wells were washed once with Dulbecco's modified Eagle's medium and the number of cells attached was estimated after lysis in 0.25% Triton X-100, and determination of the activity of hexosaminidase, as described by Landegren [16].

Determination of *N*-terminal amino acid sequences

Dissociated TPP II (69 μg in the dialysis buffer) was digested with chymotrypsin (2.6 μg) for 2 h at 30 °C. The reaction was terminated by the addition of sample buffer. The mixture was heated at 55 °C for 15 min before being loaded into ten wells on an 11% polyacrylamide gel in the presence of SDS, prepared as described above. The separated polypeptides were transferred electrophoretically to an Immobilon membrane, with 50 mM-sodium phosphate buffer, pH 6.5, as the transfer buffer. After transfer for 4 h at 400 mA and 4 °C, the membrane was stained with Coomassie Brilliant Blue, as described by Matsudaria [17]. The bands corresponding to 70 kDa, 42 kDa and 20 kDa were cut out from six lanes and analysed by gas-phase sequencing on an Applied Biosystems model 470A protein sequencer. The sequenceable amount varied between 10 and 20 pmol for the three polypeptides.

RESULTS

Cross-reactivity between TPP II and fibronectin

Polyclonal hen antibodies against human erythrocyte TPP II were affinity-purified twice, as described in the Experimental section. When tested against a human fibroblast lysate in an immunoblot analysis (Fig. 1*a*, lane 2), the antibodies reacted with a protein (135 kDa), apparently corresponding to TPP II. In addition, a broad band with molecular mass greater than 200 kDa could be seen on the immunoblot. This band co-migrated with isolated human fibronectin, which was found to react with the antibodies against TPP II (Fig. 1*a*, lane 4). The specificity of the antibody preparation was investigated by immunoblot analysis against a crude TPP II preparation (Fig. 1*a*, lane 3). The multiple immunoreactive bands with molecular mass lower than 135 kDa that can be seen in this lane and in Figs. 2 and 4 are considered to be due to partially degraded enzyme, since they can be labelled by di-[^{3}H]isopropyl phosphorofluoridate in the same way as the 135 kDa band [2]. Furthermore, it is evident from lane 8 in Fig. 1(*a*) that antibodies raised against human plasma fibronectin cross-reacted with human TPP II. The specificity of this antiserum was investigated

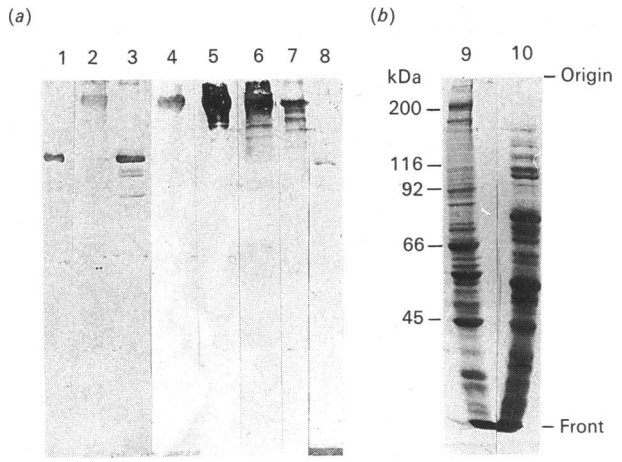

Fig. 1. Cross-reactivity between TPP II and fibronectin

PAGE (8% gel) and immunoblot analysis were performed as described in the Experimental section. The antibodies used were hen anti-(TPP II) antibodies at 0.01 mg/ml (lanes 1–4) and rabbit anti-(human fibronectin) antibodies at 2.3 mg/ml (lanes 5–8). The polyacrylamide gel was loaded with human TPP II (0.3 μg in lane 1 and 1 μg in lane 8), lysed fibroblasts (0.07 mg in lanes 2, 6 and 9), pool from DEAE-cellulose chromatography of human haemolysate [2] (0.12 mg in lanes 3, 7 and 10) or 8 μg of human fibronectin (lanes 4 and 5). (*a*) Immunoblot; (*b*) Coomassie Blue-stained polyacrylamide gel.

by immunoblot analysis against a fibroblast lysate and the crude TPP II preparation (Fig. 1*a*, lanes 6 and 7). It can be noted that, even though several immunoreactive bands can be seen, the bulk of proteins (Fig. 1*b*) were not recognized by these antibodies. It is also a fact that fibronectin is sensitive to proteolytic digestion [18], indicating that several of the observed immunoreactive bands might be degradation products. The cross-reactivity could also be detected in an immunoblot analysis with commercially available hen antibodies against human fibronectin at a protein concentration of 0.03 mg/ml (results not shown).

A false cross-reactivity might have occurred if traces of fibronectin had been present in the TPP II preparation used for immunization. However, since the 135 kDa TPP II subunit used in the affinity column was electro-eluted from a preparative SDS/polyacrylamide gel, the possibility that the affinity-purified antibody preparation would have contained any fibronectin monospecific antibodies was markedly diminished. In addition, traces of TPP II in the fibronectin preparation were looked for by immunoblot analysis (Fig. 1*a*) and enzymic activity measurement (results not shown), with negative result.

The fact that the antibodies that were bound to a fibronectin–Sepharose column recognized TPP II as well as fibronectin (Fig. 2, lanes 5 and 6) further supports the existence of cross-reactivity. Antibodies that did not bind to the column recognized only TPP II in an immunoblot analysis (Fig. 2, lanes 1–4).

Fibronectin can be divided into functional domains through limited proteolysis [19]. Thus a 105 kDa cell-binding fragment, a 40 kDa collagen-binding domain and a 29 kDa fibrin-binding domain have been isolated. Proteolytic fragments, with different fragment size, have also been used for raising antibodies [9]. In the latter case, anti-(65 kDa fragment) antibodies were antibodies against a cell-binding fragment, anti-(30 kDa fragment) antibodies were against the collagen-binding domain, anti-(31 kDa fragment) antibodies were against the heparin-binding domain and anti-(29 kDa fragment) antibodies were against the fibrin-binding domain. When antibodies against different fibronectin fragments were tested towards TPP II in an immunoblot analysis

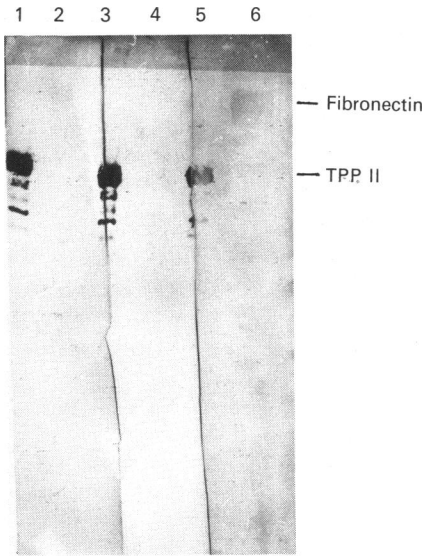

Fig. 2. Reactivity of anti-(TPP II) antibodies after affinity chromatography on a fibronectin–Sepharose column

Affinity-purified hen anti-(TPP II) antibodies (2 ml) were loaded on to a fibronectin–Sepharose column. The column was prepared and eluted as described in the Experimental section. PAGE (8% gel) and immunoblot analysis were performed as described in the Experimental section. The polyacrylamide gel was loaded with TPP II (1 μg in lanes 1, 3 and 5) and fibronectin (10 μg in lanes 2, 4 and 6). The antibodies used were: eluate from sample application (lanes 1 and 2), eluate from first wash (lanes 3 and 4) and Sephadex-G-25-chromatographed $MgCl_2$ eluate from the column (lanes 5 and 6), each at a concentration of about 0.01 mg/ml.

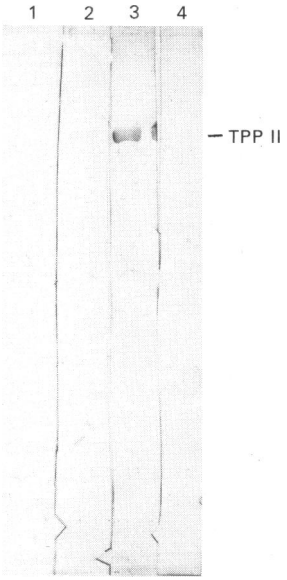

Fig. 3. Cross-reactivity between TPP II and antibodies raised against fragments of human fibronectin

PAGE (8% gel) and immunoblot analysis were performed as described in the Experimental section. The polyacrylamide gel was loaded with TPP II (1 μg in lanes 1–4). The antibodies used were anti-(29 kDa fragment) antibodies (lane 1), anti-(30 kDa fragment) antibodies (lane 2), anti-(65 kDa fragment) antibodies (lane 3) and anti-(31 kDa fragment) antibodies (lane 4), each at a concentration of 0.2 mg/ml.

(Fig. 3), apparently only the antibodies against the cell-binding fragment of fibronectin [anti-(65 kDa fragment) antibodies] reacted. Moreover, anti-(TPP II) antibodies reacted with the

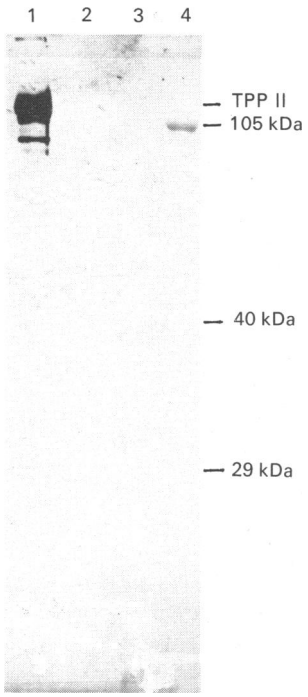

Fig. 4. Cross-reactivity between anti-(TPP II) antibodies and fibronectin fragments

PAGE (11% gel) and immunoblot analysis were performed as described in the Experimental section. The polyacrylamide gel was loaded with 2 μg of TPP II (lane 1), 29 kDa fibronectin fragment (lane 2), 40 kDa fibronectin fragment (lane 3) and 105 kDa fibronectin fragment (lane 4). The antibodies used were hen anti-(TPP II) antibodies at a concentration of 0.01 mg/ml.

105 kDa cell-binding fragment of fibronectin, but not with the 40 kDa collagen-binding domain or the 29 kDa fibrin-binding domain (Fig. 4).

Proteolytic digestion of TPP II

In order to determine what part of the large TPP II molecule is immunologically similar to fibronectin, a partial proteolytic digestion of the enzyme was performed. The ordered polymeric form of TPP II [3] seemed to be rather resistant to proteolytic digestion by chymotrypsin, even in an enzyme/substrate molar ratio of 2:1 (Fig. 5, lanes 1–4), with the exception of the material represented by the minor bands in the TPP II preparation. This material, which is believed to represent partially degraded subunits (cf. the comments on Fig. 1), was susceptible to further proteolytic attack, possibly because it is more exposed. When the large polymeric structures were dissociated into smaller units through dialysis against a dilute Tris buffer [3], the enzyme protein became more sensitive to proteolytic digestion (Fig. 5, lanes 5–8). The difference noted was not due to a major change in chymotrypsin activity, since the activity in the Tris buffer used for digestion of dissociated TPP II was 90% of that in the potassium phosphate buffer used for digestion of native TPP II (results not shown). Chymotrypsin attacked a few sensitive bonds, as inferred from the fact that three essentially stable fragments, with apparent molecular masses of 70 kDa, 42 kDa and 20 kDa, were formed.

Investigation of the TPP II fragments

To determine in which of the fragments the serine residue of the active site is located, TPP II was labelled with di-[^{3}H]isopropyl phosphorofluoridate, dialysed and digested with chymotrypsin. By this procedure the active site was found to be part of the 70 kDa fragment (Fig. 6*b*, lane 2).

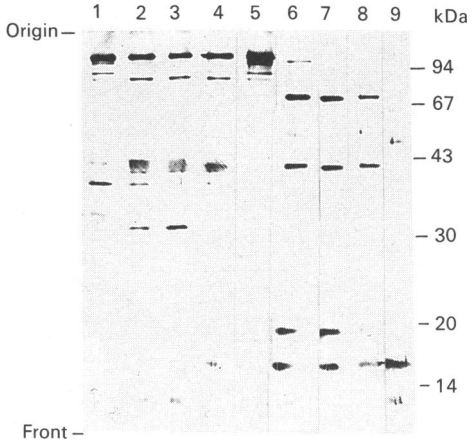

Fig. 5. Chymotryptic digestion of native and dissociated TPP II

Native TPP II (lanes 1–4) or dissociated TPP II (lanes 5–8) (1 μg) was digested with chymotrypsin (0.4 μg) in 50 mM-potassium phosphate buffer, pH 7.5, containing 30% glycerol and 1 mM-dithiothreitol (for native TPP II) or in 2 mM-Tris/HCl buffer, pH 8.0, containing 3% glycerol and 0.5 mM-2-mercaptoethanol (for dissociated TPP II) at 30 °C in a total volume of 40 μl. At the indicated times, 10 μl of the reaction mixture was removed and the digestion was terminated by the addition of sample buffer. The mixtures were treated as described in the Experimental section and loaded on an 11% polyacrylamide gel, and the electrophoresis and silver-staining were performed as described in the Experimental section. Lanes 1 and 5, no digestion; lanes 2 and 6, 10 min chymotryptic digestion; lanes 3 and 7, 30 min digestion; lanes 4 and 8, 2 h digestion; lane 9, chymotrypsin (0.1 μg).

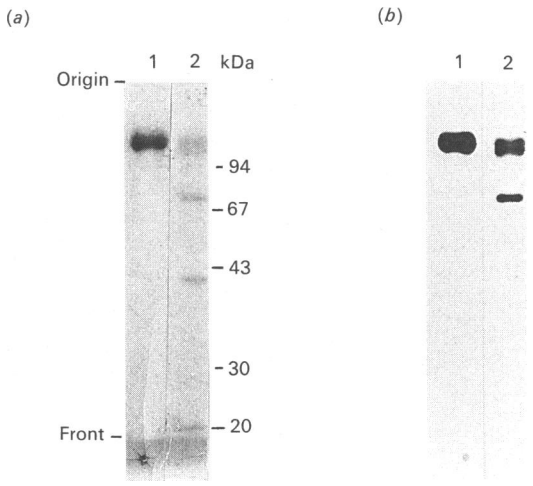

Fig. 6. Location of the active site in TPP II fragments

TPP II was labelled with 0.2 mM-di-[^{3}H]isopropyl phosphorofluoridate (0.8 μCi/nmol) in 33 mM-potassium phosphate buffer, pH 7.5, containing 20% glycerol and 1 mM-dithiothreitol for 1 h at 20 °C and thereafter dissociated by dialysis against Tris/HCl buffer as described in the Experimental section. The labelled dissociated TPP II (8 μg) was digested with chymotrypsin (0.4 μg) for 2 h at 30 °C and the digestion was terminated by the addition of sample buffer. The samples were treated as described in the Experimental section, then loaded on to an 11% polyacrylamide gel, and the electrophoresis and Coomassie Blue staining were performed as described in the Experimental section. Fluorography, with the use of Amplify, was performed as previously described [2]. The X-ray film was exposed for 14 days. Lane 1, undigested TPP II; lane 2, digested TPP II. (*a*) Polyacrylamide gel stained by Coomassie Brilliant Blue; (*b*) fluorogram.

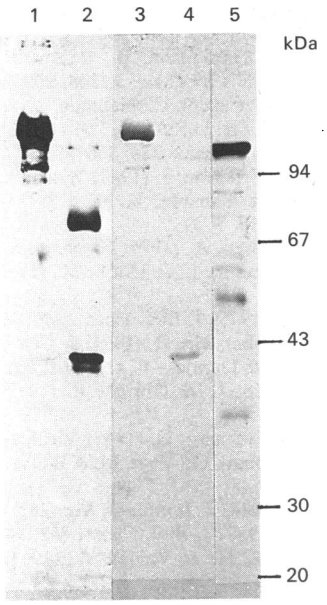

Fig. 7. Immunological reactivity towards TPP II fragments

TPP II (2 μg) was dissociated by dialysis against Tris/HCl buffer, as described in the Experimental section, then digested with chymotrypsin (0.08 μg) for 2 h at 30 °C, and thereafter loaded on to an 11% polyacrylamide gel (lanes 2 and 4). Electrophoresis and immunoblot analysis were performed as described in the Experimental section. As controls, native TPP II (2 μg) was loaded in lanes 1 and 3, and 105 kDa fibronectin fragment (2 μg) in lane 5. The antibodies used were hen anti-(TPP II) antibodies (0.01 mg/ml) (lanes 1 and 2) and anti-(65 kDa fragment) antibodies (0.2 mg/ml) (lanes 3–5).

The immunological reactivity of the TPP II fragments was investigated by immunoblot analysis (Fig. 7). Affinity-purified antibodies raised against native TPP II recognized the 70 kDa fragment and the 42 kDa fragment, but not the 20 kDa fragment. Whether this is due to lack of a sufficient number of antigenic determinants in the 20 kDa fragment, or the possibility that this fragment is not exposed during immunization, is not known. Fig. 7 also shows that the 42 kDa fragment is specifically recognized by antibodies raised against the cell-binding fragment of fibronectin. Even though this large fibronectin fragment also possesses other functions, these results prompted an investigation of the cell-binding properties of TPP II. Hepatocytes were seeded in wells coated with native or dissociated TPP II, or chymotryptic fragments thereof, as described in the Experimental section. No hepatocytes (< 5% of the fibronection control) could, however, be found in the TPP II-coated wells (results not shown). Thus no cell-binding ability of TPP II could be detected under these experimental conditions.

Determination of *N*-terminal sequences of TPP II fragments

In a preliminary attempt to investigate the possible structural similarities between TPP II and fibronectin, the *N*-terminal amino acid sequences of the TPP II fragments were determined after the fragments had been electroblotted on to Immobilon membranes [17], as described in the Experimental section (Table 1).

For the 20 kDa fragment the first 18 residues were easily identified, whereas the 42 kDa fragment was sequenced through only 14 residues. Amino acid sequence similarities between the 42 kDa fragment and human fibronectin were sought by means of the search command 'Bestfit' of the GCG program [20] on a VAX 8200 computer. The best fit showed a 46% similarity to fibronectin residues 1643–1655, i.e. His-Glu-Leu-Phe-Pro-Ala-Pro-Asp-Gly-Glu-Glu-Asp-Thr. These residues belong, however, to the extra domain of fibronectin [21], which is located between the cell-binding and heparin-binding domains of cellular fibronectin. Since this domain is not present in plasma fibronectin, this similarity should not account for the immunological cross-reactivity with TPP II. No sequence similarity could be observed between fibronectin and the *N*-terminal amino acid sequences of the 20 kDa fragment or the 70 kDa fragment.

The *N*-terminal amino acid sequence of the 70 kDa fragment (Table 1) could not be unambiguously determined beyond the ninth residue, owing to a fairly high background of, in particular, glycine and serine. The data were, however, in agreement with the previous determination of the subtilisin-like amino acid sequence of the active site [4]. It is therefore concluded that residues 3–11 of the 70 kDa fragment are identical with the amino acid sequence surrounding the active-site serine residue [4]. This result is also compatible with the finding above of a di-[^{3}H]isopropyl label in the 70 kDa fragment (cf. Fig. 6) and suggests that the active-site serine residue is located near an exposed hinge region between two domains. It may also be noted that neither the *N*-terminal amino acid sequence of the 42 kDa fragment nor that of the 20 kDa fragment displayed any sequence similarity to subtilisin.

DISCUSSION

It has been established in this paper that there is an immunological similarity between the cell-binding fragment of human fibronectin and a 42 kDa fragment of human TPP II. However, no cell-binding property of TPP II could be detected, nor could a significant structural relationship between fibronectin and the *N*-terminal part (14 amino acid residues) of the 42 kDa fragment be observed. It is therefore possible that a common antigenic determinant between TPP II and fibronectin either lies in other parts of the 42 kDa TPP II fragment or is a feature of the three-dimensional structure.

The cell-binding domain of fibronectin consists of a number of repeating units (type III repeats) [7]. These units are, however, not restricted to fibronectin, since type III repeats have also been observed in another extracellular matrix glycoprotein, cytotactin [22]. The possibility that the cross-reactivity observed between TPP II and fibronectin might be due to the existence of type-III-

Table 1. *N*-Terminal amino acid sequences of TPP II fragments

N-Terminal sequence analysis was performed as described in the Experimental section. Parentheses indicate uncertainty in the determination.

Fragment	*N*-Terminal sequence
20 kDa	Lys-Thr-Asp-Pro-Arg-Pro-Asp-Ala-Ala-Thr-Ile-Lys-Asn-Asp-Met-Asp-Lys-Gln-Xaa-Ser
42 kDa	His-Gly-Leu-Leu-Pro-Lys-Lys-Glu-Thr-Xaa-Ala-Ala-Ser-Phe
70 kDa	Arg-Gly-Thr-Gln-Leu-Met-Asn-Gly-Thr-(Ser)-(Met)

like repeats also in TPP II may therefore be considered. It has been suggested, e.g. for the serine proteinases involved in blood coagulation and fibrinolysis [23], and for immunoglobulins [24], that proteins evolve partly by combining genetic elements, which specify protein domains with different functions. If this is the case also for the large TPP II protein, the cross-reactivity between fibronectin and TPP II may imply a role for the peptidase in the extracellular matrix, in addition to its role as an intracellular peptidase.

The immunological cross-reactivity between TPP II and fibronectin, studied in this work, indicates structural similarities between the two proteins. However, more definite statements on the nature, as well as the functional implications, of such similarities will have to await the determination of the full amino acid sequence of the enzyme and the demonstration of possible similarities to the fibronectin amino acid sequence.

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