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Mapping the collagen-binding site of human fibronectin by expression in *Escherichia coli*

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The collagen-binding domain of human fibronectin has been expressed as a cro/β-galactosidase fusion protein in *Escherichia coli*. The hybrid polypeptide was recognized by an anti-(human plasma fibronectin) serum and bound specifically to gelatin-Sepharose. The collagen-binding region was subdivided by constructing a series of overlapping bacterial expression plasmids. The fusion proteins produced by these constructs were analysed for gelatin-binding activity. The results indicate that the binding site lies within an ~12.5 kd fragment of fibronectin, and show that the following 14 amino acid sequence is critical for gelatin-binding activity: Ala-Ala-His-Glu-Glu-Ile-Cys-Thr-Thr-Asn-Glu-Gly-Val-Met. This sequence links the second type II homology unit with the adjacent type I repeat in the amino-terminal third of the fibronectin molecule.

Key words: bacterial expression/collagen-binding/fibronectin

Introduction

Fibronectin is a high mol. wt glycoprotein present in blood plasma, extracellular matrices and at cell surfaces. It has been implicated in a variety of contact processes, including cell attachment and migration, opsonization and wound healing (for reviews see Hynes and Yamada, 1982; Furcht, 1983). The fibronectin molecule is a disulphide-linked dimer and demonstrates a number of subunit variants with mol. wts of 230-250 kd. These variants are generated by complex alternative splicing of the primary transcript of a single gene (for reviews see Hynes, 1985; Owens *et al.*, 1986). Each subunit of fibronectin contains specific sites for binding to cells and a range of macromolecules including collagens, fibrin and heparin. Reflecting its multiple roles, fibronectin shows a modular organization comprising three different internal homology repeats (types I, II and III; Petersen *et al.*, 1983). These units are arranged in a highly conserved pattern which correlates with the location of the binding sites along the length of the molecule.

With the exception of the cell-binding site which has been mapped to a tetrapeptide sequence (RGDS; Pierschbacher and Ruoslahti, 1984), very little is known about the exact structure of the other binding sites of fibronectin. Of these, the collagen-binding site is particularly important. The interaction between fibronectin and collagen appears to be fundamental to the organization of extracellular matrices and the behaviour of cells on these substrates (Vaheri *et al.*, 1978; McDonald *et al.*, 1982; Nagata *et al.*, 1985). This interaction also forms the basis of the standard procedure for purifying fibronectin in which gelatin (denatured collagen) linked to Sepharose is used as an affinity matrix (Engvall and Ruoslahti, 1977). The collagen-binding domain of plasma fibronectin from different species has been variously isolated as a 30-45 kd mol. wt proteolytic fragment (e.g. human, Gold *et al.*, 1979; and Ruoslahti *et al.*, 1979; rat, Balian *et al.*, 1979; bovine, Skorstengaard *et al.*, 1982). It is characterized by the presence of the only type II internal repeats in the molecule, in addition to a number of type I units (Skorstengaard *et al.*, 1984). The unique occurrence of the type II units together with their high level of amino acid conservation implicates these sequences in collagen-binding. The recent availability of cDNA clones covering this region in human fibronectin (Kornblihtt *et al.*, 1985) enables this to be tested directly. Here we describe the mapping of the collagen-binding site of human fibronectin using bacterial expression constructs. We show that the binding site lies well within the region defined by proteolytic cleavage of fibronectin and indicate the involvement of a specific amino acid sequence located between the second type II unit and adjacent type I unit.

Results

Construction and characterization of fibronectin expression plasmids

The human fibronectin cDNA clones, pFH134, pFH16 and pFH6, encompass all or part of the collagen-binding domain of fibronectin identified by proteolytic cleavage of the protein (Figure 1). These cDNAs were therefore chosen as the starting point for investigating the expression of a functional collagen-binding site in *Escherichia coli*. The pEX vectors used for cloning enable exogenous gene sequences to be inserted into a polylinker in all three reading frames at the 3′ end of a cro-LacZ hybrid gene under the control of the λ P_r promoter (Stanley and Luzio, 1984). The 5′ ends of the 1.74, 1.04 and 1.3 kb inserts of pFH134, pFH16 and pFH6 respectively were sequenced (Maxam and Gilbert, 1980) to establish the reading frames of the cDNAs and blunt-end cloned into the *Sma*I site of either pEX2 (pFH134, pFH16) or pEX1 (pFH6). The recombinant plasmids were introduced into an *E. coli* strain previously transformed with a plasmid encoding the temperature-sensitive λ P_r repressor, cI587. This allows for temperature-inducible expression of the cro/β-galactosidase protein. To test for the production of fibronectin fusion proteins by the expression constructs, hybridization-positive clones were grown at 30°C for 2.5 h and then shifted to 42°C for a further 2 h. Total bacterial lysates were analysed by SDS-polyacrylamide gel electrophoresis. Five of ten pXFH134 constructs, one of seven pXFH16 and one of eight pXFH6 constructs showed the production of high mol. wt polypeptides of sizes consistent with the lengths of the cDNA inserts (~185, ~165 and ~170 kd respectively, Figure 2a, tracks 1, 2 and 3). The sense and anti-sense strands of the sequences introduced into the pEX plasmids were analysed for theoretical translation products in all three reading frames. The in-phase fusion of β-galactosidase and fibronectin gave the only full length open reading frame. The correct orientation of the fibronectin sequences in the three pXFH constructs was also confirmed by restriction enzyme digests.

The fusion proteins produced by pXFH134, pXFH16 and pXFH6 accounted for ~5-10% of the total bacterial protein consistent with the previous report for this vector system (Stanley

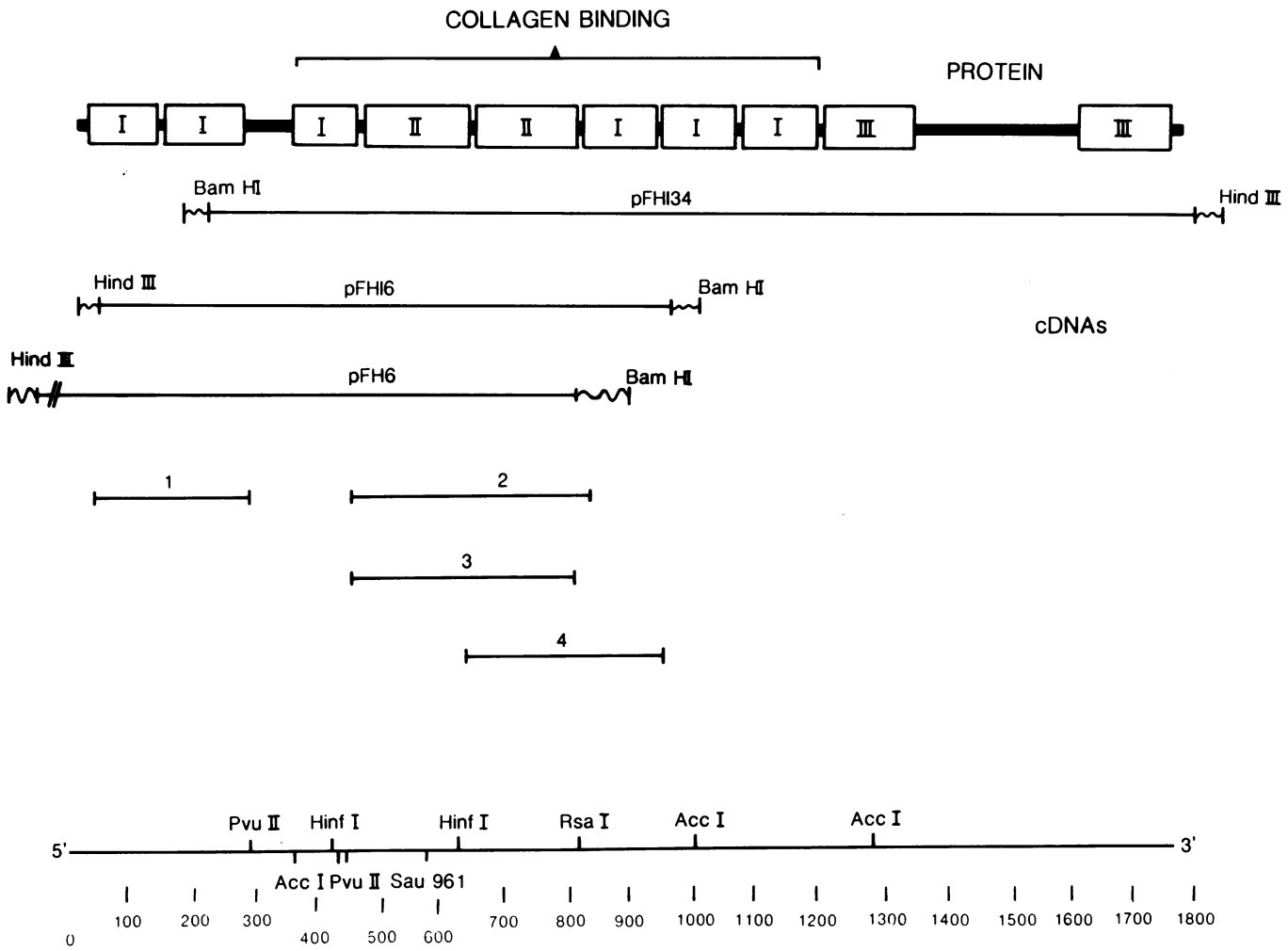

Fig. 1. Map of the human fibronectin cDNAs expressed in *E. coli*. A schematic diagram of part of the fibronectin protein is shown indicating the position of the collagen-binding domain and internal homologies (I, II and III). Below, the position and sizes (bp) of the series of overlapping fibronectin cDNAs used in the bacterial expression experiments is indicated. Only the positions of the restriction enzyme sites used in the isolation of cDNAs 1–4 are shown. The flanking *Hin*dIII and *Bam*HI sites of pFH134, pFH16 and pFH6 occur in the polylinker of the vector.

and Luzio, 1984). All the fusion proteins showed some proteolytic degradation particularly the pXFH134 and pXFH6 polypeptides, which appeared to be partially cleaved to the size of the wild-type cro/β-galactosidase (cro/β-gal 116 kd, Figure 2, tracks 1, 3 and 4). Analysis of proteins synthesized over a time course of induction (10–120 min) indicated that proteolysis occurred concomitantly with synthesis of the fusion proteins (data not shown).

The expression of fibronectin antigenic determinants by the pXFH plasmids was investigated by immunoprecipitation from biosynthetically labelled cell lysates using a rabbit polyclonal anti-(human plasma fibronectin) serum. An anti-(*E. coli* β-galactosidase) serum was used as a control. The anti-(fibronectin) serum precipitated the 185 kd polypeptide synthesized by pXFH134 but not the pXFH16 and pXFH6 fusion proteins or the cro/β-galactosidase polypeptide (Figure 2b, tracks 9–12). By contrast all the polypeptides produced by the pEX plasmids reacted with the anti-(β-gal) serum (Figure 2b, tracks 5–8). The results indicate that the epitope(s) recognized by the antibody to fibronectin lie outside the type II homology units and adjacent type I repeats (Figure 1). This observation is consistent with the poor antigenicity of the collagen-binding domain of human fibronectin previously reported (Ruoslahti *et al.*, 1979) and most probably reflects the very high level of amino acid conservation in this region (Figure 4). The results with the anti-(β-gal) serum confirmed the identity of the 116 kd band among the products of pXFH134 and pXFH6 as the cro/β-gal polypeptide.

Gelatin–Sepharose affinity chromatography

The pXFH134 plasmid covers the entire collagen-binding domain of fibronectin and was tested first for gelatin-binding activity. Overproduction of β-galactosidase fusions in *E. coli* results in the precipitation of the proteins in the cells as insoluble inclusion bodies (Williams *et al.*, 1982a; Cheng, 1983; Stanley, 1983). Therefore, when bacteria expressing the pXFH134 plasmid were lysed by sonication and centrifuged, the fibronectin fusion protein was found exclusively in the insoluble pellet. This fraction represented ~50% of the total protein of the bacterial lysate. Solubilization of this material required treatment with 7 M urea and, following dialysis, 60% of the protein remained in solution. The dialysate, which was highly enriched in the fusion protein, was applied directly to a 5 ml gelatin–Sepharose column equilibrated in 50 mM Tris-HCl, pH 7.4. The column was washed with 0.5 M NaCl in 50 mM Tris-HCl, pH 7.4 until the E_{280} of the flow-through was < 0.01. The cro/β-gal–fibronectin hybrid protein was eluted from the column as a single symmetrical peak

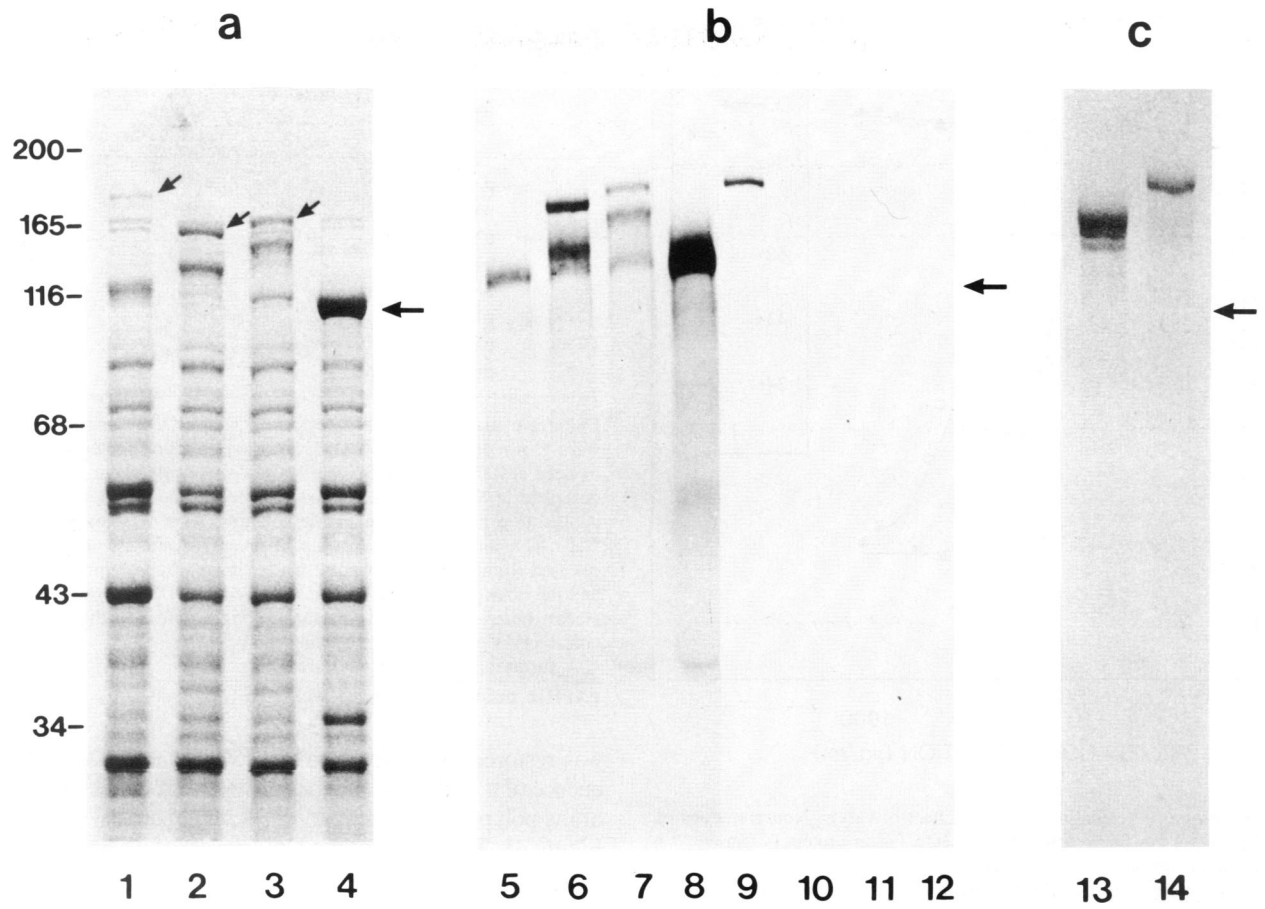

Fig. 2. Analysis of cro/β-galactosidase−fibronectin fusion proteins. The expression of the fusion proteins produced by pXFH134 **(tracks, 1, 5, 9** and **14)**, pXFH16 **(tracks 2, 6, 10** and **13)** and pXFH6 **(tracks 3, 7** and **11)** and pEX2 vector only **(tracks 4, 8** and **12)** was induced at 42°C. All samples were analysed on 7.5% polyacrylamide gels in the presence of SDS under reducing conditions. The positions of the fusion polypeptides and cro/β-gal are marked by arrows. The numbers refer to mol. wt × 10^{-3} kd. **(a)** Total protein profiles of *E. coli* expressing hybrid plasmids. Polypeptides were stained with Coomassie blue. **(b)** Immunoprecipitation of ^{35}S-methionine-labelled (100 μCi/ml for 30 min) bacterial lysates with either anti-(*E. coli* β-galactosidase) serum **(tracks 5−8)** or anti-(human fibronectin) serum **(tracks 9−12)**. **(c)** Elution of fusion proteins from gelatin−Sepharose with 4 M urea. Eluted fractions were combined, concentrated by precipitation with trichloroacetic acid (12.5% w/v) and equal amounts of protein loaded onto the gel. Polypeptides were stained with Coomassie Blue.

with 4 M urea in the same buffer (Figure 2, track 14). Under these conditions fibronectin is specifically released from gelatin−Sepharose (Ruoslahti *et al.*, 1982). None of the wild-type cro/β-gal protein present in the lysate of pXFH134 cells was retained by the column (compare Figure 2, tracks 1 and 14). Similarly, in the control experiment using pEX2 only, no significant binding of the cro/β-gal protein to the gelatin−Sepharose was observed. In a parallel experiment, neither the pXFH134 nor pEX2 polypeptides bound to unsubstituted Sepharose under the same conditions.

The fibronectin fusion proteins produced by pXFH16 and pXFH6 were then tested for gelatin-binding activity. pXFH16 fusion polypeptide was bound to gelatin and specifically eluted with urea, whereas pXFH6 showed no significant binding (Figure 2c, track 13; inset Figure 3). Two to three times more pXFH16 bound to the column than pXFH134, consistent with the lower level of fusion protein degradation in pXFH16 compared with pXFH134 (Figure 2a). It must be noted, however, that the fusion proteins specifically eluted from the gelatin−Sepharose column represented < 5% of those applied to the column. Application of the throughflow of the column back into the gelatin−Sepharose matrix gave no further binding, indicating that the binding capacity of the matrix was not limiting. In addition, reapplication of the specifically eluted fraction to the gelatin−Sepharose column resulted in complete re-binding. This material could again be recovered by urea treatment. Thus, although binding activity is obtained from the bacterial lysates, considerable activity is lost due to the insolubilization of the fusion proteins in the bacterial cells, and the subsequent vigorous treatment required to resolubilize them.

Collectively the results indicate that a functional collagen-binding site has been reconstituted in the cro/β-gal fibronectins. From the sequences covered by the constructs, it appears that the binding site lies within the domain defined at the protein level (Figure 1). Furthermore, the observation that pXFH16 binds to gelatin but that pXFH6 does not, indicates that the gelatin-binding activity involves sequence(s) coded for by the 3′ 2.5 kb of pXFH16. Before analysing this in more detail an important question is whether the gelatin-binding activity of the cro/β-gal− fibronectins expressed in *E. coli* corresponds to that of intact fibronectin. The pXFH16 protein was used to investigate this question in two ways. Firstly the ability of the fusion protein to compete with fibronectin in binding to gelatin was tested. As shown in Figure 3, the pXFH16 polypeptide inhibited the binding of [^{125}I]fibronectin to gelatin though at a higher concentration than native fibronectin. Secondly the effect of disrupting disulphide bonds was analysed. The type I and II repeats that make up the collagen-

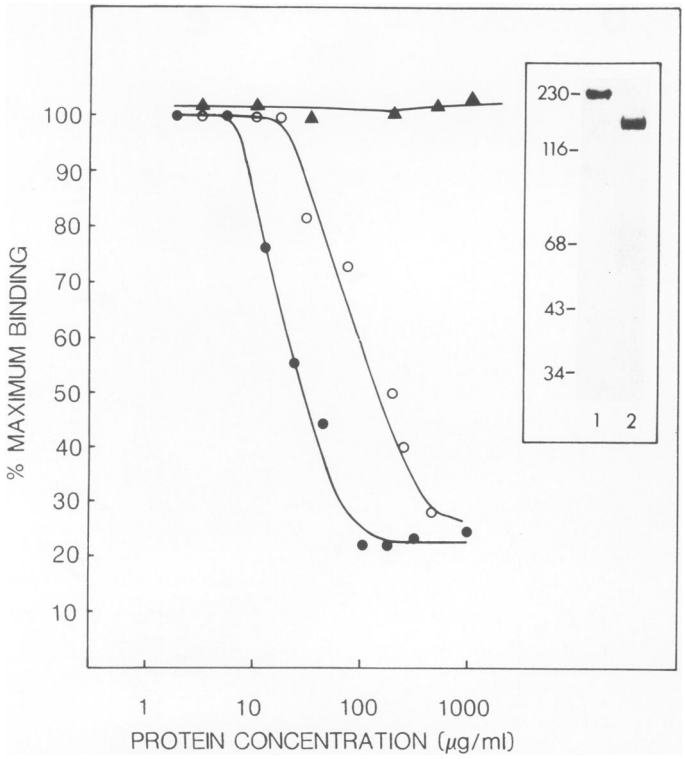

Fig. 3. Inhibition of gelatin-binding. The effect of varying concentrations of either fibronectin (● inset; **track 1**); pXFH16 fusion protein (○ inset; **track 2**); or bovine serum albumin (▲) on the binding of [^{125}I]fibronectin to gelatin was assayed as described in Materials and methods. The results are expressed as a percentage of the radioactivity bound in the absence of competitor proteins.

binding domain of fibronectin are each characterized by two intrachain disulphide bridges. The maintenance of these linkages is necessary for the gelatin-binding activity of fibronectin (Balian *et al.*, 1979). Reduction/carboxymethylation of the pXFH16 protein and fibronectin completely abolished their gelatin-binding activities compared with untreated controls (data not shown). Thus the pXFH16 construct directs the synthesis of a polypeptide that folds to form an authentic collagen-binding site with identical specificity to native fibronectin.

Localization of the collagen-binding site

The carboxy terminus of the pXFH6 polypeptide, which does not bind to gelatin, coincides exactly with the end of the second type II homologous repeat of fibronectin. Whereas pXFH16, which does bind gelatin, covers in addition to the type IIs the adjacent one and a third type I units, implicating these latter sequences in gelatin-binding (Figure 1). To test this possibility and thus further localize the binding site four cro/LacZ−fibronectin hybrids were constructed using available restriction enzyme sites (Figure 1). The fusion proteins synthesized from these plasmids were tested for gelatin-binding activity by affinity chromatography. The results are summarized in Table I. pXFN1 was a control and showed no binding. The pXFN4 polypeptide bound specifically to gelatin as predicted. The level of binding was approximately the same as for pXFH16 indicating that the combining site is localized in an ~12.5 kd fragment consisting essentially of a complete type II and type I repeat. The two type II repeats of fibronectin alone appear to be insufficient to specify gelatin-binding since the pXFN3 polypeptide did not bind to gelatin−Sepharose. However, most interestingly gelatin-binding activity

Table I. Relative gelatin-binding activities of the fusion proteins produced by the pEX fibronectin constructs

pEX construct	Binding to gelatin−Sepharose
pXFH134	0.53
pXFH16	1.0
pXFH6	0.0
pXFN1	0.0
pXFN2	0.54
pXFN3	0.0
pXFN4	1.1
pEX2 vector only	0.0

Cultures of bacteria (1 litre) transformed with pEX fibronectin constructs were grown for 2.5 h at 30°C. Expression of fusion proteins was then induced at 42°C for 2 h. Soluble lysates of the cells were prepared as described in Materials and methods and equal amounts of total protein applied to 5 ml gelatin−Sepharose columns. After washing with 0.5 M NaCl, columns were eluted wtih 4 M urea. The amount of fusion protein released from gelatin−Sepharose was estimated by absorption at 280 nm and the homogeneity of the eluates verified by SDS−polyarylamide gel electrophoresis. The relative gelatin-binding activities of the different pEX constructs is given by the following ratio:

$$\frac{\text{fusion protein eluted with urea } (E_{280} \text{ units})}{\text{pXFH16 fusion protein eluted with urea } (E_{280} \text{ units})}$$

was restored to this segment to ~50% that of pXFH16, if a sequence of a further 14 amino acids was included at the C terminus of the polypeptide (Figure 1 pXFN2 compared with pXFN3 and Figure 4). The 3′ end of the DNA insert of pXFN2 is arbitrarily defined by an *Rsa*I site. However the position of this restriction site in the DNA sequence coincides with the end of a short stretch of non-homologous amino acid sequence between the second type II unit and adjacent type I (Figure 4). The results strongly suggest that this sequence is critical for the collagen-binding function of fibronectin.

Attempts to define the collagen-binding site further using shorter fibronectin cDNAs (< 200 bp) in the fusion constructs proved inconclusive. Thus polypeptides produced by constructs comprising individual type II or type I units did not show significant gelatin-binding even if the 14 residue sequence, implicated above, was included in the cDNAs. It appears that the large cro/β-gal polypeptide may interfere with the accurate folding of these shorter sequences. Therefore a non-fusion expression system, for example in yeast, would be required to extend these studies.

Discussion

This study was undertaken to investigate the location of the collagen-binding site in fibronectin using a bacterial expression system. This binding site has been mapped by proteolytic cleavage of fibronectin to an ~45 kd domain (Figure 4) close to the NH_2 terminus of the protein. cDNA fragments covering this region in human cellular fibronectin were expressed in *E. coli* as cro/β-galactosidase gene fusions. By assaying the expressed hybrid proteins for gelatin-binding activity, the fibronectin binding site for collagen was localized to an ~12.5 kd fragment (amino acids 373−508, Figure 4) in human fibronectin. With the exception of two amino acid changes (residues 477 Ile for Val and 490 Asp for Gly), this sequence is identical to that in bovine plasma fibronectin (Skorstengaard *et al.*, 1984). Further, evidence was obtained that a stretch of 14 amino acids within this fragment is critical for the binding specificity. Whether this represents part

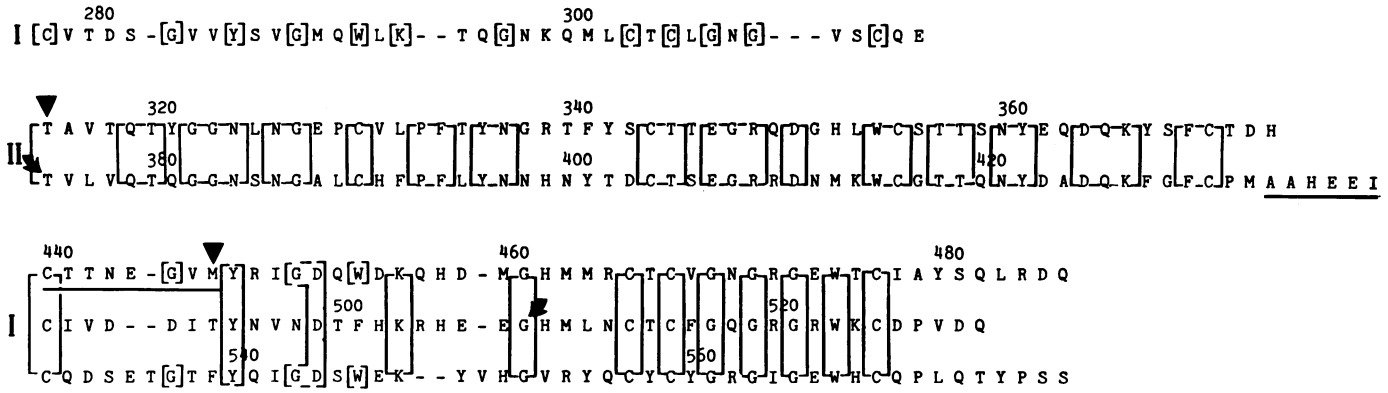

Fig. 4. Amino acid sequence of the collagen-binding domain of human fibronectin. The human fibronectin sequence that corresponds to the 45 kd collagen-binding fragment of bovine fibronectin is shown (Skorstengaard *et al.*, 1984). Arrows mark the positions of the inserts of pXFN2 (▼) and pXFN3 (▼). The specific amino acid sequence implicated in collagen-binding is underlined. Identical residues in the type I and II homologies are boxed. Amino acids are numbered from the NH_2 terminus of the mature protein.

or all of the actual collagen-binding site is still an open question. The results do not rule out a role for other sequences in the 12.5 kd fragment (or even outside it) in collagen-binding. However, the data allow the importance of some specific sequences to be assessed. Thus it has been shown that the type II homology units that are unique to the collagen-binding domain of fibronectin (Figure 4) are alone insufficient to specify the binding activity. However, they may contribute to the binding site in terms of the secondary structure of the region. The collagen-binding activity of fibronectin is dependent upon the integrity of disulphide linkages in the molecule. There are two such bonds in each of the type II (and type I) repeats.

The mechanism of the binding of fibronectin to collagen is not understood, though the interaction results in a significant conformational change in the fibronectin polypeptide (Williams *et al.*, 1982b). Fibronectin binds most efficiently to type III collagen of the different genetic types of the protein, and in all cases binds denatured collagens with higher affinity than the native forms (Engvall *et al.*, 1978). This suggests that the tertiary structure of collagens is not necessary for their recognition by fibronectin, though it may modulate the interaction.

Experiments with purified collagen chains or CNBr fragments indicate that fibronectin recognizes a specific region in the α_1 chain of collagen that is close to the collagenase cleavage site (Kleinman *et al.*, 1976; Dessau *et al.*, 1978). The nature of this interaction is unclear. The resistance of collagen–fibronectin binding to high salt concentrations appears to rule out the involvement of electrostatic forces (Ruoslahti and Engvall, 1978; Ruoslahti *et al.*, 1982; Vuento *et al.*, 1982; Forastieri and Ingham, 1985). The fact that fibronectin is released from gelatin–Sepharose by chaotropic agents or polyethylene glycol has been taken to indicate the involvement of hydrophobic interactions (Ruoslahti and Engvall, 1978). However, contradicting this, it has been shown that collagen–fibronectin binding is pH-dependent and sensitive to ionic (e.g. deoxycholate) but not non-ionic detergents (e.g. Triton X-100) (Vuento *et al.*, 1982; Forastieri and Ingham, 1985). In addition, Forastieri and Ingham (1985), analysing the fluid-phase interaction between gelatin and fluorescein-labelled fibronectin showed that binding involved an entropy change which is not usually associated with hyrophobic interactions. These authors favour hydrogen-bonding as the basis of collagen–fibronectin binding. In this context, the sequence implicated in collagen-binding in the present report is largely composed of hydrophilic amino acids, including a number of residues that could be involved in hydrogen-bonding (Figure 4). Experiments are in progress to define the collagen-binding site further and to investigate the importance of specific amino acids in the interaction.

Materials and methods

Antisera

An anti-(bacterial β-galactosidase) serum was kindly provided by Dr I.Jones and an anti-(human plasma fibronectin) serum was a gift from Dr D.Critchley (Department of Biochemistry, Leicester University, Leicester, UK).

Preparation of recombinant plasmids

The isolation and characterization of the human fibronectin cDNA clones, pFH6, pFH16 and pFH134 has been described previously (Kornblihtt *et al.*, 1985). For subcloning into pEX vectors, restriction fragments were filled in with the Klenow fragment of DNA polymerase I and blunt-end ligated into *Sma*I cut/phosphatased pEX1, 2 or 3. Transformations were carried out using the *E. coli* strain LK111 (Zabeau and Stanley, 1982) harbouring the plasmid pcI857 which specifies kanamycin resistance and carries the cI857 allele (Remaut *et al.*, 1983). Colonies were transferred to Whatman 541 filter paper (Gergen *et al.*, 1979) and screened with either 3′ end-labelled (Maxam and Gilbert, 1977) or nick-translated probes (Rigby *et al.*, 1977). Plasmids were designated as pXFH6, 16, 134 and pXFN1–4.

Preparation of bacterial extracts

Bacteria carrying recombinant plasmids were grown at 30°C for 2.5 h and expression of the cro/β-galactosidase fusion protein induced by shifting to 42°C for 2 h. Bacteria were pelleted at 1200 *g* and washed with 50 mM Tris-HCl, pH 7.4, 170 mM NaCl. Cells were resuspended in the same buffer containing lysozyme (2.5 mg/ml) and sonicated for 2 min on ice. The lysate was centrifuged at 45 000 *g* for 30 min at 4°C. The pellet was resuspended in 7 M urea in 10 mM Tris-HCl, pH 7.4, 1 mM EDTA and incubated at room temperature for 30 min. The solubilized extract was dialysed extensively against 50 mM Tris-HCl, pH 7.4, at 4°C and then centrifuged at 45 000 *g* for 30 min at 4°C.

Gelatin-binding experiments

Gelatin–Sepharose was either obtained from Sigma Chemicals (St. Louis, MO, USA) or prepared by linking gelatin (pig skin type I, Sigma Chemicals) to CNBr-activated Sepharose CL.4B (Pharmacia, Uppsala, Sweden). Chromatography of bacterial extracts on gelatin–Sepharose was carried out as described by Ruoslahti *et al.* (1982). The efficacy of the gelatin–Sepharose matrix was verified using purified human plasma fibronectin (Sigma Chemicals).

For the inhibition test, wells in a microtitre plate were coated with 0.1 μg/ml gelatin. Two-fold serial dilutions of fibronectin or cro/β-gal–fibronectin were made in 10 mM Na phosphate buffer containing 150 mM NaCl (PBS), 0.05% (v/v) Tween 20 and 3% (w/v) bovine serum albumin. Plates were incubated for 4 h at 20°C and then 0.04 μCi[^{125}I]fibronectin (440 Ci/mmol; NEN Research Products, FRG) added to each well. After a further 4 h incubation, wells were washed with the diluting buffer and counted for radioactivity.

Other procedures

To prepare biosynthetically labelled bacterial cell extracts, 1 ml cultures were grown for 2 h at 30°C and then induced for 30 min at 42°C. Cells were pelleted into 1 ml PBS and incubated for a further 30 min with 10 μCi[^{35}S]methionine

(600 Ci/mmol; Amersham International, UK). Soluble protein extracts were prepared by lysing cell pellets in 0.5% (w/v) SDS for 10 min at 60°C. The extracts were diluted with 10 mM Tris-HCl buffer, pH 7.4, containing 1% (v/v) NP-40, 1 mM EDTA, 150 mM NaCl, 1 mg/ml bovine serum albumin and 1 mM phenyl methyl sulphonyl fluoride and centrifuged at 12 000 *g* for 5 min. Immunoprecipitation from cell lysates was carried out as described by Owen *et al.* (1980), using Protein A–Sepharose to precipitate antigen–antibody complexes.

SDS–polyacrylamide gel electrophoresis was carried out in 0.1% (w/v) SDS in Tris/glycine buffer on 7.5% (w/v) acrylamide slab gels (Laemmli, 1970). Gels were stained with 0.1% Coomassie blue in methanol:water:acetic acid (4:5:1 by vol).

Protein was estimated by the method of Bradford (1976) using bovine serum albumin as a standard.

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References

Balian,G., Click,E.M., Crouch,E., Davidson,J.M. and Bornstein,P. (1979) *J. Biol. Chem.*, **254**, 1429–1432.

Bradford,M. (1976) *Anal. Biochem.*, **72**, 248–254.

Cheng,Y. (1983) *Biochem. Biophys. Res. Commun.*, **111**, 104–111.

Dessau,W., Adelmann,B., Timpl,R. and Martin,G.R. (1978) *Biochem. J.*, **169**, 55–59.

Engvall,E. and Ruoslahti,E. (1977) *Int. J. Cancer*, **20**, 1–5.

Engvall,E., Ruoslahti,E. and Miller,E.J. (1978) *J. Exp. Med.*, **147**, 1584–1595.

Forastieri,H. and Ingham,K.C. (1985) *J. Biol. Chem.*, **260**, 10546–10550.

Furcht,L. (1983) *Modern Cell Biol.*, **1**, 53–117.

Gergen,J.P., Stern,R.H. and Wensink,P.C. (1979) *Nucleic Acids Res.*, **7**, 2115–2136.

Gold,L.I., Garcia-Pardo,A., Frangione,B., Franklin,E.C. and Pearlstein,E. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 4803–4807.

Hynes,R.O. (1985) *Annu. Rev. Cell Biol.*, **1**, 67–90.

Hynes,R.O. and Yamada,K.M. (1982) *J. Cell. Biol.*, **95**, 369–377.

Kleinman,H.K., McGoodwin,E.B. and Klebe,R.J. (1976) *Biochem. Biophys. Res. Commun.*, **72**, 426–432.

Kornblihtt,A.R., Umezawa,K., Vibe-Pedersen,K. and Baralle,F.E. (1985) *EMBO J.*, **4**, 1775–1779.

Laemmli,U.K. (1970) *Nature*, **227**, 680–685.

Maxam,A.M. and Gilbert,W. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 560–569.

Maxam,A.M. and Gilbert,W. (1980) *Methods Enzymol.*, **65**, 499–580.

McDonald,J.A., Kelley,D.G. and Broekelmann,T.J. (1982) *J. Cell. Biol.*, **92**, 485–492.

Nagata,K., Humprhies,M.J., Olden,K. and Yamada,K.M. (1985) *J. Cell. Biol.*, **101**, 386–394.

Owen,M.J., Kissonerghis,A.-M. and Lodish,H. (1980) *J. Biol. Chem.*, **255**, 9678–9684.

Owens,R.J., Kornblihtt,A.K. and Baralle,F.E. (1986) In McLean,N. (ed.), *Oxford Surveys on Eukaryotic Genes*. Oxford University Press, Vol. 3, in press.

Petersen,T., Thøgersen,H.C., Skorstengaard,K., Vibe-Pedersen,K., Sahl,P., Sottrup-Jensen,L. and Magnusson,S. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 137–141.

Pierschbacher,M.D. and Ruoslahti,E. (1984) *Nature*, **309**, 30–33.

Remaut,E., Tsao,H. and Fiers,W. (1983) *Gene*, **22**, 103–113.

Rigby,P.W.J., Dieckmann,H., Rhodes,C. and Berg,P. (1977) *J. Mol. Biol.*, **113**, 237–251.

Ruoslahti,E. and Engvall,E. (1978) *Ann. N.Y. Acad. Sci.*, **312**, 178–191.

Ruoslahti,E., Hayman,E.G., Kuusela,P., Shively,J.E. and Engvall,E. (1979) *J. Biol. Chem.*, **254**, 6054–6059.

Ruoslahti,E., Hayman,E.G., Pierschbacher,M. and Engvall,E. (1982) *Methods Enzymol.*, **82**, 803–831.

Skorstengaard,K., Thøgersen,H.C., Vibe-Pedersen,K., Petersen,T.E. and Magnusson,S. (1982) *Eur. J. Biochem.*, **128**, 605–623.

Skorstengaard,K., Thøgersen,H. and Petersen,T. (1984) *Eur. J. Biochem.*, **140**, 235–243.

Sottrup-Jensen,L. and Magnusson,S. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 137–141.

Stanley,K.K. (1983) *Nucleic Acids Res.*, **11**, 4077–4092.

Stanley,K.K. and Luzio,P. (1984) *EMBO J.*, **3**, 1429–1434.

Towbin,H., Stahelin,T. and Gordon,J. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 4350–4354.

Vaheri,A., Kurkinen,M., Lehto,V.-P., Linder,E. and Timpl,R. (1978) *Proc. Natl. Acad. Sci. USA*, **75**, 4944–4948.

Vuento,M., Salonen,E., Osterlund,K. and Stenman,U.-H. (1982) *Biochem. J.*, **201**, 1–8.

Williams,D.C., Van Frank,R.M., Muth,W.L. and Burnett,J.P. (1982a) *Science* **215**, 687–689.

Williams,E., Janmey,P.A., Ferry,J.D. and Mosher,D.F. (1982b) *J. Biol. Chem.*, **257**, 14973–14978.

Zabeau,M. and Stanley,K.K. (1983) *EMBO J.*, **1**, 1217–1224.

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Note added in proof

A monoclonal antibody (D96) has recently been raised against the 45 kd collagen-binding domain of human fibronectin and has been shown to inhibit the binding of fibronectin to collagen [Hasty *et al.*, (1986) *J. Cell Sci.*, **81**, 125–142]. We have localized the epitope recognized by this antibody, to the 12.5 kd collagen-binding fragment of fibronectin, described in this report.