All Six Modules of the Gelatin-binding Domain of Fibronectin are Required for Full Affinity

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Abstract

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The gelatin-binding sites of fibronectin are confined to a 42 kDa region having four type I and two type II modules in the following order I₆-II₁-II₂-I₇-I₈-I₉. To determine the relative importance of each module for recognition of gelatin, recombinant green fluorescent fusion proteins were prepared in which individual modules or groups of modules were deleted and the resulting proteins were tested for binding to gelatin by analytical affinity chromatography. Deletion of both type II modules did not eliminate binding confirming that at least some of the type I modules in this region are able to bind gelatin. It was found that deletion of type I module 6 tends to increase the affinity whereas deletion of any other module decreases it. Deletion of module I₉ has a large effect but only if module II₂ is also present, suggesting an interaction between these two noncontiguous modules. Analysis of more than 20 recombinant fusion products leads to the conclusion that all modules contribute to the interaction, either directly, by contacting the ligand, or indirectly through module-module interactions.

Introduction

Fibronectin is a large modular glycoprotein found in the extracellular matrix and body fluids of higher organisms. It interacts with a variety of other macromolecules including integrin receptors, heparan sulfate proteoglycans, fibrin, tenascin and several types of denatured collagens (gelatins). Here we focus on the interaction with gelatin. The gelatin-binding sites of fibronectin are confined to a region having four type I and two type II modules in the following order I₆-II₁-II₂-I₇-I₈-I₉ (Fig. 1). This region can be isolated as a 42 kDa fragment (42kDa GBF) that binds to gelatin with affinity only slightly lower than that of the parent protein (1). Fragments outside this region including an N-terminal 29k fragment (type I modules 1 through 5) and a C-terminal 19k fragment (type I modules 10 through 12) do not bind gelatin. The 42kDa fragment contains the only type II modules in fibronectin, modules that are thought to play a role in binding to gelatin, not only in fibronectin but in other gelatin-binding proteins, especially some matrix metalloproteinases where type II modules are also found (2). However, the 42kDa GBF can be further cleaved into two non-overlapping subfragments, I₆-II₁-II₂-I₇ and I₈-I₉, both of which also bind gelatin, albeit with ~ 10 fold lower affinity than the parent fragment (3). This suggests that type II modules are not essential for binding of fibronectin to gelatin, although they may contribute, and that some type I modules can bind. It was recently reported that a recombinant 2-module fragment II₁-II₂ bound weakly to gelatin but that the
affinity was about 5 fold higher in a 3-module fragment I₆-II₁-II₂ (4). The latter fragment was shown in the same study to have a hairpin-like structure in which module I₆ interacts strongly with II₂ in spite of the intervening II₁. This highlights the fact that affinity for gelatin may be affected not only by the presence of a given module but by the interaction of that module with neighboring modules.

In the present study we have prepared a large number of recombinant fragments in which one or more modules have been deleted from 42kDa GBF in order to further understand the importance of all the modules for binding to gelatin. It is shown that modules II₂ and I₉ are most critical for binding but that all six modules contribute directly or indirectly to the binding site.

Materials and Methods

Materials: Polyclonal anti-green fluorescent protein (GFP) antibody was purchased from Clontech. Spodoptera frugiperda (Sf9) cells adapted to grow in serum-free medium Sf900-II were obtained from Invitrogen. Swine skin gelatin (type I) was from Sigma. The 42-, 30-, and 21-kDa gelatin-binding fragments were purified from a thermolysin digest of human plasma fibronectin as described previously (5).

Plasmid constructs: A vector (pENTR11-MHG) encoding a secretion signal, (His)₆-, and green fluorescent protein (GFP)- tags at the N-terminus was described previously (6). cDNAs encoding the gelatin binding domain of rat fibronectin and its variants were amplified by pfu DNA polymerase (Stratagene), followed by BamHI-HindIII digestion, and then subcloned into BglIII-HindIII sites of pENTR11-MHG. The primers used for amplification are given in Table 1 where amino acids are numbered from the amino terminus (pyroglutamic acid) of the processed fibronectin. The inserts were transferred to pDEST8 (Invitrogen) using the Gateway cloning system (Invitrogen).

Preparation of recombinant fragments: Recombinant fragments were expressed in SF9 insect cells using the Bac-to-Bac expression system (Invitrogen). A bacterial strain (DH10Bac) was transformed with recombinant plasmids and colonies containing recombinant bacmids were grown for isolation of the resulting viral DNA according to the manufacturer's instructions.
Supernatants of transfected Sf9 cells were used to infect more Sf9 cells through two or three stages of amplification. Sf9 cells (2 x 10^8) were then infected with recombinant viruses, and supernatants were harvested 48 or 72 h after infection. Recombinant GFP-Fn fragments were purified on Talon metal affinity resin (Clontech), eluting with 150 mM imidazole, and then dialysed against phosphate buffered saline (PBS, pH 7.4). The identity of each fragment was verified by detection of the appropriate viral DNA in the infected cells with PCR (O'Reilly et al., 1992) and by the apparent molecular weight of the purified protein on SDS-PAGE. Yields varied between 0.5 and 1.2 mg per 100 ml of culture supernate.

**Gelatin affinity chromatography:** Analytical affinity chromatography was performed as described previously using an FPLC system (Pharmacia) (7). Briefly, recombinant protein was injected onto a gelatin-Sepharose column equilibrated with PBS and pumped at a flow rate of 1 ml/min. After washing, a linear gradient of urea (0 – 6 M) was applied between 10 and 30 minutes and the bound GFP-fusion proteins were detected with a fluorescence monitor (Shimadzu Model RF535). In some experiments, unbound and bound fractions were collected and subjected to immunoblot analysis using anti-GFP antibody (8). The variable amount of unbound green fluorescence in the analytical runs is due to varying degrees of proteolytic cleavage at the flexible linker between GFP and the GBFs during handling and/or storage of the samples.

**Solid-phase binding assay:** Ninety-six well microtiter plates were coated with 10 µg/ml gelatin in PBS for 16 h at 4°C. After washing and blocking with 1% BSA in PBS, the plates were incubated with various concentrations of recombinant proteins for 2 h at room temperature. Plates were washed three times with 0.2% BSA in PBS and then incubated with polyclonal anti-GFP antibody for 1 h at room temperature, followed by incubation with horseradish peroxidase-conjugated donkey anti-rabbit IgG antibody for 40 min at room temperature. After three washes with PBS, plates were developed with TMB Microwell peroxidase substrate (KPL). Absorbance was measured with a Dynatec plate reader and C(50%) values were obtained by fitting the data to a simple binding isotherm using Sigmaplot.

**Results**

Since each of the six modules in the 42kDa gelatin-binding fragment of fibronectin has a unique number, 6,1,2,7,8 and 9, those numbers will be used as a short hand notation to specify the
modular composition of the various mutants and dashes (−) will be used to designate deletions. Various combinations of these modules were expressed as fusion proteins with enhanced green fluorescence protein (GFP) using a baculovirus expression system. Initially, we used gelatin-Sepharose in conjunction with SDS-PAGE and immunoblotting to assess binding. Typical results are shown in Fig. 2 where it can be seen that the pair of type II modules alone does not bind to gelatin-Sepharose whereas a fragment containing type I modules 7, 8 and 9 does bind. These data suggest that type II modules alone are neither sufficient nor essential for binding to gelatin and that at least some type I modules are able to bind without the help of type II modules.

**Analytical affinity chromatography** on gelatin-Sepharose was used to rank the relative affinities of the various fusion proteins, using green fluorescence to monitor elution in a urea gradient. The results for all products are presented in Fig. 3, where panel A refers to the wild type fusion protein, designated 612789. Its peak elution occurs at 3.3 M urea, very close to that observed with natural 42kDa GBF as previously reported (6). This elution profile is reproduced as a dotted line in all other panels for easy comparison. Except for those panels where no peak is evident, the profiles have been normalized to give the same amplitude at their peak elution. The urea concentrations at peak elution are summarized in Table 2.

The next five panels, B through G, show results for all single module deletions, from amino to carboxyl terminus respectively. Although none of these deletions abolishes binding, all of them have a measurable effect on the elution position. Interestingly, removal of module 6 actually increases the concentration of urea required for elution to about 3.8 M (panel B). The other deletions shift the elution to lower urea in varying degrees, loss of module 7 having the smallest effect and module 9 the largest. These results alone lead to the conclusion that all six modules play a role, directly or indirectly, in binding to gelatin.

Panels G, H and I show the effects of successive deletions from the carboxyl terminal end. Once module 9 has been removed, further deletion of module 8 has very little effect, as if its only role is to tether 9 in the appropriate position. Further deletion of module 7 yields a three module fragment, 612−−, which fails to bind to gelatin Sepharose, although slight retardation is evident. This lack of binding of 612−− was confirmed in a separate experiment using an
immunoblotting approach like that in Fig. 2 (data not shown).

Panels J, K and L represent successive deletions of modules from the amino terminal end. As noted above, deletion of module 6 increases the affinity (panel J is identical to panel B). Further deletion of module 1 seems to reverse the effects of deleting module 6, so that the elution of the four module fragment, $\text{---2789}$, is indistinguishable from that of the full-length fragment. The next deletion produces the three module fragment $\text{---789}$ which in spite of its lack of type II modules, is able to bind (as in Fig. 2), albeit with lower affinity than $\text{---2789}$. This proves again that type II modules are not essential for binding of fibronectin to gelatin although the second one has some effect.

In panel M are results for a four-module fragment lacking both terminal type I modules, 6 and 9. It was shown above (panel G) that deletion of module 9 causes the largest decrease in affinity of any single module deletion while deletion of module 6 (panel B) caused an increase in affinity. Here it is seen that the enhancing effect of deleting module 6 also occurs upon its removal from $\text{61278-}$, shifting the peak elution from 2.15 to 2.45 M urea, and partially compensating for the effect of removing module 9.

Deletion of either terminal module 1 or 8 from $\text{-1278-}$ had very little further effect (panels N and O) but deletion of both to produce the bimodular fragment $\text{--27--}$ (panel P) caused a noticeable weakening with substantial amounts of protein bleeding from the column during the wash phase, prior to application of the urea gradient. Module 7 binds slightly better when partnered with module 8 (panel R) than with module 2 (panel P). Panel Q shows that a lone pair of type II modules ($\text{-12--}$) does not bind tight enough to survive the wash phase, consistent with its absence in the 6th lane of Fig. 2. However, deletion of this type II pair from the full length fragment to yield $\text{6--789}$ significantly lowers the affinity (panel S).

Fragment $\text{--2789}$ is the only four-module fragment that binds with full affinity. Removal of either of its terminal modules substantially lowers that affinity by a similar amount (panels K, L and N). However, deletion of the interior modules 7 and 8 from $\text{--2789}$ generates a bimodular fragment containing only 2 and 9, which failed to bind with significant affinity although
retardation was evident (panel T). If modules 7 and 8 in either or in the full length fragment are replaced by homologous modules 4 and 5 from outside the gelatin binding region, the binding is essentially abolished (panels U and V). The effect of deleting modules 7 and 8 from the full length fragment (panel W) is much less severe than replacing them with modules 4 and 5. That modules 7 and 8 serve more than a spacer function is evident from the fact that they can bind independently as a pair (panel R).

From the foregoing results it is apparent that all six modules in the gelatin-binding domain of fibronectin play a role in ligand recognition, either directly through contact with gelatin or indirectly by influencing neighboring modules.

**ELISA Analysis** was used as an alternative measure of the affinity of some of the fusion proteins for gelatin. Plastic microtitre wells were coated with gelatin, incubated with increasing concentrations of the fusion protein and binding was detected immunologically with antibody to green GFP. Representative data are shown in Fig. 4. The concentrations required for half-saturation ($C_{50\%}$) in this and other experiments not shown varied between 0.2 and $\sim$5 µM, as summarized in Table 2. The range of values of $C_{50\%}$ obtained for the full-length fragment is in good agreement with $K_d$ values between 0.6 and 1.2 µM determined previously in the fluid phase (3, and references therein). In Fig. 5, the values of $C_{50\%}$ are plotted against the urea concentration required for peak elution. In spite of considerable scatter in the data, there is a reasonable correlation. Most of the scatter can be attributed to uncertainties in the $C_{50\%}$ values determined by ELISA, which is not the optimum method for weak interactions. The affinity chromatography results, although only qualitative, are quite reproducible and thus provide a more reliable determination of the rank order of affinities.

The difference in urea concentration required for elution of any mutant relative to the full length fragment, calculated from the data in column 3 of table 2, or for any pair of mutants differing by a single module, is summarized visually in Fig. 6, where results are grouped according to the module that was deleted. Note how module 6 stands out from the others in that its deletion consistently enhances binding whereas most of the others, especially 1 and 9, have a weakening effect when deleted.
Discussion

The results presented here indicate that recognition of gelatin is a robust property of fibronectin. Any one of the six modules within the 42 kDa gelatin-binding domain (612789) can be deleted without abrogation of binding. At the same time, all six modules appear to play some role since each of their deletions has a measurable effect on affinity. Thus the situation is more complicated than is often found with domain deletion experiments of this type. For example, the fact that fragment --2789 binds with the same affinity as the full length fragment would suggest that modules 6 and 1 are not important. But this conclusion is rendered invalid by the observation that removal of 6 alone actually increases the affinity while removal of 2 alone decreases it by a similar degree. When both are removed the effects appear to compensate.

There are two ways in which a given module might contribute to the interaction with gelatin. The first is direct, i.e., the module engages the ligand, and the other is indirect, i.e., the module interacts with and affects the conformation or disposition of neighboring modules that actually make contact. Some modules could operate at both of these levels. The three dimensional structure of the trimodular fragment I_6II_1II_2 (Fig.1) shows that modules need not be contiguous to engage in such interactions; I_6 forms an extensive interface with II_2 despite the intervening II_1(4). The carboxyl terminus of II_2 in that structure is located such that the following module, I_7, could also interact with I_6, as was inferred from earlier observations that the thermal stability of the latter was greater in fragments where the former was present (5). Thus, there is reason to suspect that the gelatin-binding domain of fibronectin is folded back upon itself providing numerous opportunities for interactions between noncontiguous modules such that the deletion of any one of them could perturb the overall arrangement of the others.

Additional evidence for long range interaction between modules comes from examination of the effect of deleting module 9. As shown in Fig. 6, deletion of this module from 612789, --12789 or --2789 consistently reduces the affinity for gelatin by a similar amount; the $\Delta[\text{urea}]$ values vary between -1.1 and -1.25 M (Fig. 6). The fact that this effect is lost in fragment ---789 could suggest some type of interaction between modules 2 and 9 that is important for binding. It should also be mentioned that deletion of modules could allow new interactions.
between other modules. For example, a weak interaction between modules 1 and 2 that was observed in the isolated pair (9) was not present in a larger fragment that also contained module 6 (4).

Replacement of type I modules 7 and 8 with type I modules 4 and 5 from the N-terminal region abolishes binding, both in the full length fragment and in --2789. This is consistent with modules 7 and 8 serving as more than mere spacers, as further evidenced by the fact that the pair alone is able to bind, even better than the pair of type IIIs. Modules 4 and 5 constitute a rigid pair with an extensive interface (10). Whether a similar interaction exists between modules 7 and 8 remains to be determined. There is no such interaction between type I modules 1 and 2 in the fib-1 region of fibronectin (11). If the connection between modules 7 and 8 is flexible, then replacing them with modules 4 and 5 may introduce constraints that prevent other interactions that are important for the overall structure and for binding. This is consistent with the fact that simply deleting modules 7 and 8 had less effect than replacing them with 4 and 5.

Module 6 stands out as the only one whose deletion actually enhances binding (Fig. 6). Note however that the enhancing effect is greatly diminished in fragments that lack module 2. This suggests that the above mentioned interaction between these modules 2 and 6, which is well documented in the known structure of 612 (Fig. 1), imposes a constraint on the overall structure that slightly impedes the interaction with gelatin. This is opposite to what was reported by the ones who determined that structure. Pickford et al. (4) reported that both 612--- and --12--- bound to gelatin Sepharose and provided evidence from surface plasmon resonance measurements to suggest that removal of module 6 from 612--- increased the affinity towards immobilized α(1) chain of human type I collagen approximately 10-fold. The discrepancy could relate to the different species of collagen and fibronectin used in the two studies.* The Kₐ values that were estimated from limited SPR data by Pickford et al (4), are designated by + symbols in our Fig. 5. Their position on the urea axis would be consistent with the failure of our corresponding fragments, if they had similar affinities, to bind to our gelatin-

** We routinely use swine skin gelatin for affinity chromatography and the recombinant fragments used in the current study were encoded by cDNA from rat fibronectin. The full length fragment behaves identical to natural 42kDa GBF derived from plasma fibronectin. The species of gelatin used by Pickford et al (4) was not specified. We also find that the affinity of 42 kDa GBF for α chains and CNBr fragments of bovine type I collagen (ref 12 and
Sepharose.

A recent report from this laboratory highlighted the fact that each of the α chains in type I collagen contains multiple sites with similar affinity for 42kDa GBF (12). The nature of those sites remains unclear. Since it is denatured collagen, i.e., gelatin, that is recognized by fibronectin, it is assumed that what is being recognized is some linear sequence as opposed to a well-defined tertiary structure. As of yet the relevant sequence has not been defined, i.e., there is no synthetic collagen-like peptide that has been shown to bind fibronectin with significant affinity. It is not even certain whether the various active subfragments of 42kDa GBF, such as those described here, are all recognizing the same sequence in gelatin. It is conceivable that neighboring sequences of the collagen chains interact with different parts of 42kDa GBF. This would provide additional opportunities for module-module interactions to play a role in defining the binding site. The present analysis of more than 20 recombinant fusion products clearly illustrates that all six modules in 42kDa GBF contribute to its interaction with gelatin, either directly, by contacting the ligand, or indirectly through module-module interactions. Determining which is which will probably require a high-resolution structure of a complex between GBF and an appropriate segment of gelatin.

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Table 1. Summary of primers used for the various GFP fusion proteins.

| Modular Composition | AA # | Primers used Forward | Reverse | Internal |
|---------------------|------|----------------------|---------|----------|
| 612789              | V243-L570 | 1                   | 2       |          |
| 61278               | V243-I526 | 1                   | 3       |          |
| 612--               | V243-L482 | 1                   | 4       |          |
| 612---              | V243-A433 | 1                   | 5       |          |
| -12789              | E312-L570 | 6                   | 2       |          |
| --2789              | V376-L570 | 7                   | 2       |          |
| ---789              | M431-L570 | 8                   | 2       |          |
| --1278              | E312-I526 | 6                   | 3       |          |
| --278               | V376-I526 | 7                   | 3       |          |
| ---78               | M431-I526 | 8                   | 3       |          |
| --27--              | V376-L482 | 7                   | 4       |          |
| -127--              | E312-L482 | 6                   | 4       |          |
| -12--               | E312-A433 | 6                   | 5       |          |
| 6--789              | V243-E326 and A432-L570 | 1       | 2       | 9        |
| 6-2789              | V243-E326 and V374-L570 | 1       | 2       | 10       |
| 61-789              | V243-H372 and A433-L570 | 1       | 2       | 11       |
| 612-89              | V243-A432 and Q481-L570 | 1       | 2       | 12       |
| 6127-9              | V243-S480 and Q528-L570 | 1       | 2       | 13       |
| 612-9               | V243-I437 and C529-L570 | 1       | 2       | 14       |
| --2--9              | E312-I437 and C529-L570 | 7       | 2       | 14       |
| 612459              | V243-H434, A152-L244, and Q528-L570 | 1       | 2       | 15 & 16  |
| --2459              | V376-H434, A152-L244, and Q528-L570 | 7       | 2       | 15 & 16  |

| Primer No. | Primer Sequence |
|------------|-----------------|
| 1          | 5'-AAGGATCCGG AGTTCACAG AGTGCTTCAC CTGG-3 |
| 2          | 5'-AAAAGCTTCT AGAGCAGAGG CTGGCAGTGC CACTCCC-3 |
| 3          | 5'-AAAAGCTTCT AGAGGATGGG GTCACATTTC CATCTGC-3 |
| 4          | 5'-AAAAGCTTCT AGAGCTGGGA GTAGGGGATG CAGG-3 |
| 5          | 5'-AAAAGCTTCT AGAGGCGACC CATTGGGCAG AATCC-3 |
| 6          | 5'-AAGGATCCGG AGAGACAGCT GTGACCCAGA CTTACG-3 |
| 7          | 5'-AAGGATCCGG AGTTCAGACT CGAGGTGGTA ATTCC-3 |
| 8          | 5'-AAGGATCCGG AGTTCACAG CTGGCAGGCA CAGG-3 |
| 9          | 5'-GCCAATCTCC TCATGGGCAG CCTCCCCITT TGAGTTGCC-3 |
| 10         | 5'-GCCAATCTCC CCTCCCCITT TGAGTTGCC-3 |
| 11         | 5'-GCCAATCTCC GCCCAATAGC CTCCCTCGCT TGAGTTGCC-3 |
| 12         | 5'-GCCAATCTCC GCCCAATAGC CTCCCTCGCT TGAGTTGCC-3 |
| 13         | 5'-GCCAATCTCC GCCCAATAGC CTCCCTCGCT TGAGTTGCC-3 |
| 14         | 5'-GCCAATCTCC GCCCAATAGC CTCCCTCGCT TGAGTTGCC-3 |
| 15         | 5'-GCCAATCTCC GCCCAATAGC CTCCCTCGCT TGAGTTGCC-3 |
| 16         | 5'-GCCAATCTCC GCCCAATAGC CTCCCTCGCT TGAGTTGCC-3 |
Table 2. Summary of binding parameters for Green GBFs

| TRACE (Fig. 3) | FRAGMENT | [UREA] AT PEAK ELUTION FROM GELATIN SEPHAROSE | C_{50\%} (µM), BY ELISA |
|----------------|----------|-----------------------------------------------|------------------------|
| A              | 612789   | 3.3                                           | 0.25-0.69              |
| B              | -12789   | 3.7                                           | 0.27-0.62              |
| C              | 6-2789   | 2.3                                           | .49-1.2                |
| D              | 61-789   | 2.6                                           | .73-1.8                |
| E              | 612-89   | 3.0                                           | .79-.81                |
| F              | 6127-9   | 2.35                                          | .51-.61                |
| G              | 61278-   | 2.15                                          | 1.4-2.5                |
| H              | 612--    | 2.15                                          | 3.2-4.7                |
| I              | 612---   | No binding                                    | 32*                    |
| J (B)          | -12789   | 3.7                                           | 0.27-0.62              |
| K              | --2789   | 3.35                                          | 0.22-0.53              |
| L              | ---789   | 2.2                                           | Not measured           |
| M              | -1278-   | 2.45                                          | 2.2-3.5                |
| N              | --278-   | 2.25                                          | 1.7-4.3                |
| O              | -127--   | 2.5                                           | 2.7-7.4                |
| P              | --27--   | 1.95                                          | 3.1-6.0                |
| Q              | -12---   | No binding                                    | 330*                   |
| R              | ---78-   | 2.25                                          | Not measured           |
| S              | 6--789   | 2.25                                          | Not measured           |
| T              | --2--9   | retarded                                      | Not measured           |
| U              | --2459   | no binding                                    | Not measured           |
| V              | 612459   | no binding                                    | Not measured           |
| W              | 612--9   | 2.8                                           | 1.7-3.4                |

The letters in the first column refer to the panels in Fig. 3 from which the data in column 3 are derived. Column 4 presents the range of C_{50\%} values obtained in at least two independent ELISA experiments. Values designated with * correspond to K_d values reported by Pickford et al.(4).
Figure Legends

Figure 1. Schematic illustration of the modular composition of human plasma fibronectin and its gelatin binding region. Type I modules are shown as ovals, type II as circles and type III as squares; the small diamonds designate glycosylation sites. The gelatin-binding domain has modular composition I6-II1-II2-I7-I8-I9, referred to herein as 612789. Also shown is a rendition of the structure of subfragment I6-II1-II2 illustrating the folding back of module II2 on to I6 (4).

Figure 2. SDS-PAGE and immunoblotting of recombinant GFP fusion proteins containing the 42kDa gelatin binding fragment of fibronectin, designated 612789 and two of its subfragments, −12--- and ---789. Lanes 1; starting material, lanes 2; unbound fraction, lanes 3; bound fraction.

Figure 3. Analytical affinity chromatography of recombinant GFP fusion proteins on gelatin-Sepharose. The numbers in each panel specify the modular composition of the gelatin-binding part of the fusion proteins. Protein solutions were applied to the column, washed with buffer and then eluted with a gradient of urea between 0 and 6 M as shown on the top scale. Elution was monitored by means of the green fluorescence. The area of the non-binding peak varies due to the varying amounts of free green fluorescent protein in the different samples, due to proteolytic degradation. The profile obtained with the full-length gelatin-binding domain is reproduced as a dotted line in each panel for easy comparison.

Figure 4. Binding of GFP fusion proteins to solid-phase gelatin as determined by ELISA. The modular compositions of the proteins were as follows from top to bottom: 6127–9, 612789, −12789, 61–789, 612–9, 61278–, −27--, and 6127--. The C50% values obtained by fitting these and other curves not shown are summarized in Table 2.

Figure 5. Correlation between C50% values obtained by ELISA and the concentrations of urea required for peak elution of the fusion proteins from gelatin-Sepharose as in Fig. 3. The straight line represents the best fit to a straight line. The + symbols represent C50% values corresponding to Kd’s reported by Pickford et al (4)
Figure 6. Summary of the effects of each module on the affinity for gelatin. The effects of deleting a given module (indicated at the right) from various parent fragments (indicated at the left) on the peak elution position in the urea gradient (Fig. 3, Table 2) are grouped according to the module that was deleted.
Fig. 1
Fig. 2
Fig. 3
Fig. 4
Fig. 5

[Graph showing Urea concentration (y-axis) vs. Log C(50%) on the x-axis. The graph includes a data set plotted as black dots and a line of best fit.]

Log C(50%)
Fig. 6
All six modules of the gelatin-binding domain of fibronectin are required for full affinity

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