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Actions of the functional upstream domain of protein F1 of *Streptococcus pyogenes* on the conformation of fibronectin

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Abstract

Fibronectin (Fn), discovered by Harvard's Plasma Protein Program as plasma "cold-insoluble globulin" in the 1940s, has attracted much interest over the past three decades. One of the most interesting features of Fn is its ability to change shape in response to various environmental conditions and interactions with other substances found in the extra-cellular space. Here we examine the potential of the functional upstream domain (FUD) of *Streptococcus pyogenes* protein F1 to bring about changes in structure of Fn. In particular, we investigate the accessibility of Fn's 10th type III module that contains the integrin binding RGD motif. By use of monoclonal antibodies in a competitive ELISA assay, we found that FUD interacts with the amino-terminal type I modules of Fn to unveil the cell-binding region of Fn. This conformational change was achieved at sub-equimolar ratios of FUD/Fn monomer. We discuss the functional relevance of the interaction for both Fn and *S. pyogenes* and correlate the results with a conformational model of Fn that arose out of a collaboration between our laboratory and that of John Ferry.

Keywords: Fibronectin; Protein F; FUD; John Ferry; Cell-binding domain; Conformational change

1. Introduction

Fibronectin (Fn) is a 500-kDa glycoprotein dimer found in its soluble form in the blood plasma at concentrations near 1 μM and in its insoluble form as a part of the extra-cellular matrix (ECM). Plasma Fn was first described as "cold-insoluble globulin" by Morrison et al. [1] of the Plasma Protein Program at the Department of Physical Chemistry, Harvard Medical School, where John Ferry began his pioneering work on fibrin assembly. Fn, as a component of the ECM, impacts important cellular activities such as migration, proliferation, differentiation, and apoptosis [2,3]. Each Fn monomer is comprised of repeating homologous sequences known as type I, II, and III modules. Dimerization occurs via disulfide bonds at the extreme C-terminus of each monomer. The nine type I modules are about 45 residues long and contain a pair of disulfide bonds. The two type II modules are about 60 residues long and again contain two stabilizing disulfide bonds The majority of Fn consists of 15–17 type III modules that contain about 90 residues and no disulfide bonds [4–6]. Thus, Fn has been likened to a "string of beads," the "beads" being the 52–56 modules in each Fn dimer [7]. Fn is readily cleaved into functional domains by controlled proteolysis [8]. These functional domains retain binding activity for cells, ECM proteins and bacteria [9].

Interestingly, proteolytic cleavage of Fn sometimes unveils cryptic binding sites and activities [10–12]. Changes in Fns structure/function, however, are not limited to proteolytic cleavage. Fn can exist in many conformations

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depending on its environment. Mechanical forces can also be used to change Fns function [13,14]. A variety of techniques have shown that the modules of Fn can adopt compact and extended conformations. Compact forms of Fn are highly convoluted and fold upon themselves [15]. Type III modules 12–14 have been shown to interact with type III modules 2–3 to help stabilize compact structures [16]. In addition to unraveling of module–module interactions, the beta-strands of individual type III modules, which lack stabilizing disulfides, can unravel increasing the overall length of Fn and potentially exposing cryptic binding sites within the protein [17–19]. These cryptic sites may allow Fn to interact with different molecules and increase its avidity for others including integrins.

It is not uncommon for bacterial pathogens to exploit Fn as a means to adhere to, colonize, and invade host tissue [20]. Among these is *Streptococcus pyogenes*, a group A streptococcus (GAS) that causes a variety of infections of the skin and soft tissue including impetigo, erysipelas, cellulitis, necrotizing fasciitis, and toxic shock syndrome [21]. GAS is capable of inducing its own entry into several eukaryotic cell types in vitro [22–25]. Entry of GAS into cells also occurs in vivo and may contribute to bacterial avoidance of antibiotic therapy [26,27].

Many bacteria express cell surface proteins known as microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) [20]. These MSCRAMMs serve to link bacterial cells to host cells through ECM components including Fn. Active sequences in MSCRAMMs tend to be disordered and only acquire order upon binding to their target matrix molecules [28]. Protein F1 (prtF1) [29] is the most efficiently used MSCRAMM on *S. pyogenes*. PrtF1's structure typifies the organization of Fn binding proteins found in other bacteria such as *Staphylococcus aureus* [30]. It contains an amino-terminal signal sequence, which targets it to the plasma membrane of the bacterium, followed by two types of repeated domains (RD1 and RD2), and a non-repetitive upstream fn binding domain (UFBD) [31]. Two functional Fn binding domains exist within the residues of the UFBD and RD2s. The first is known as the functional repeated domain (FRD) and includes 44 residues of two contiguous RD2 repeats beginning and ending with the motif MGGQSES [32]. The second, known as the functional upstream domain (FUD), includes the 43 residues of the UFBD and the amino-terminal six residues of the first RD2 [32]. This sequence interacts specifically with the amino-terminal modules of Fn [33] in a way that blocks deposition of Fn into ECM [34].

Here we show, using monoclonal antibodies in a competitive ELISA assay, that Fn, upon binding FUD, undergoes a conformational change unveiling the 10th type III module. Since the module contains the integrin binding RGD motif and based on the importance of interactions with cells, this finding has implications for the physiology of both Fn and *S. pyogenes*.

2. Materials and methods

2.1. Materials

Bovine serum albumin (BSA) and Tris were from Sigma (St. Louis, MO). Salts were from Fisher (Hampton, NH). Human plasma Fn was purified as described previously [35]. Hybridoma cells expressing mAbIII-10 (clone 5A11) previously described by Ugarova et al. [36] were a generous gift from Ed Plow, Cleveland Clinic Research Foundation. 9D2, an antibody to Fn's first type III module, was generated in our laboratory [37]. Protein concentrations were determined by absorbance at 280 nm using extinction coefficients calculated from primary sequences.

2.1.1. Recombinant protein production

Because the pUR-4 plasmid [32] contains a cysteine residue that causes dimerization of FUD [33], a thrombin cleavage site was introduced into the plasmid encoding the FUD peptide between the amino-terminal His-tag and the beginning of the FUD peptide sequence. This was accomplished with using the following primers: 5′ -AAAA GGA TCC TTA GTG CCT CGC GGA AGC-3′ ; and 5′ -TTTT GGT ACC CTT GCT TCC GCG AGG CAC-3′ . The amplified product was digested with *Kpn*I and *Bam*HI and inserted into appropriately digested pQE-30 expression vector (Qiagen, Valencia, CA). FUD was purified on a Ni^{2+} resin as reported previously [32]. Once purified, the His tag was removed from FUD by thrombin cleavage. Briefly, three units of biotinylated thrombin (EMD Biosciences, Madison, WI) were added per mg peptide diluted in thrombin cleavage buffer (20 mM Tris–HCl, pH 8.4, 150 mM NaCl, 2.5 mM $CaCl_2$). Cleavage was allowed to proceed for 16 h at room temperature in the presence of 0.05% sodium azide. After the cleavage reaction, the biotinylated thrombin was captured by incubating the reaction mixture twice (30 min at room temperature) with streptavidin agarose (EMD Biosciences) (20 μl/unit of thrombin). A final incubation with Ni-NTA agarose (Qiagen) (150 μl resin/mg peptide) allowed for the removal off the His-tag fragment and any uncleaved peptide.

Fn previously cloned into the baculovirus transfer vector pAcGP67A.coco [38] was used as template for PCR-based mutagenesis to remove the amino-terminal modules and create a truncated molecule. A segment of DNA encoding for Fn starting at the first type III module, residue 578 (Ser), through the fourth type III module, residue 968 (Thr) was amplified. The 5′ primer (AT CCA CCC GGG AGT GGT CCT GTC GAA GTA TTT ATC AC) added the restriction site *Xma*I. The 3′ primer (GT TGT CTG TTG AGC AGT CAG AGG C) is located just down stream from a *Nco*I site in the Fn sequence. This PCR product was digested with *Xma*I and *Nco*I, and ligated into the Fn.coco plasmid digested with these same enzymes. The resulting construct, FNIII1-C EDa+.coco, which starts at Ser 578 and ends at the natural C-terminus (plus a small linker and a six His tag)

and contains the alternatively spliced EDa type III module and the 89-residue version of the V-region (V89). In order to create a protein more similar to plasma Fn, which lack the EDa module, FNIII1-C EDa+.coco and DNA encoding Fn without the EDa region [39] were digested with *Eco*RI. The *Eco*RI fragment from the Fn without the EDa region was ligated into the FNIII1-C construct resulting in FNIII1-C EDa-.coco. Recombinant protein was produced using recombinant baculovirus and purified as described [38].

2.1.2. Competitive inhibition ELISA

Competitive inhibition ELISAs were done in a similar fashion as described previously [36]. Fn was mixed with various concentrations of FUD in TBS-T (10 mM Tris, pH 7.4, 50 mM NaCl+0.05% Tween-20) for 30 min at room temperature. mAbIII-10 or 9D2 was added to dilutions which made it the limiting component in the assay immediately before replicate aliquots (50 μl) were transferred to PRO-BIND™ 96 well assay plates (Beckton Dickinson, Franklin Lakes, NJ) which had been coated with 2 mg/ml Fn overnight at 4 °C, and blocked with 5% non-fat dried milk+1%BSA for 1 h at room temperature. The plates were incubated with primary antibody mixtures for 1.5 h at room temperature and washed four times with TBS-T. After washing, alkaline phosphatase conjugated donkey anti mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) was added (50 μl) was added and allowed to incubated for 1 h at room temperature. The plate was washed again four times in TBS-T prior to the addition of 50 μl Sigma 104® phosphatase substrate (*p*-Nitrophenyl Phosphate, disodium salt) (Sigma-Aldrich) diluted to 1 mg/ml in TBS pH 9.0. Color was allowed to develop at room temperature and quantified at 405 nm on an EL 340 Bio Kinetic Microplate Reader (Bio Tek Instruments, Winooski, VT).

3. Results

The "string of beads" structure of Fn evident early on by electron micrographs of Fn and corroborated by John Ferry's analysis of the persistence length of Fn [40] has the potential to facilitate interactions with other ECM proteins, bacteria, and cells. Cellular interactions occur between integrin receptors and several subsites within Fn [41]. Like many functions of Fn, cell binding can be regulated through structural changes. It has been shown, using competitive ELISAs, that the adsorption of Fn to surfaces, incubation of soluble Fn in high-salt, or interaction of soluble Fn with heparin, heparan sulfate, gangliosides, or gelatin modulates the expression of the cell-binding domain including the RGDS containing 10th type III module [36]. Heparin, gangliosides, and gelatin all interact with the amino-terminal modules of Fn [9]. We therefore examined the potential of the FUD of prtF1 to unveil the 10th type III module in a similar fashion. Fig. 1 shows that increasing concentrations of Fn compete with Fn deposited in microtiter wells for mAbIII-10 binding. Preincubation of Fn with FUD greatly enhances the competitive ability of Fn, FUD alone had no effect on the binding of mAbIII-10 binding to deposited Fn. These results suggest that FUD alters the conformation of Fn in such a way that reveals the 10th type III module.

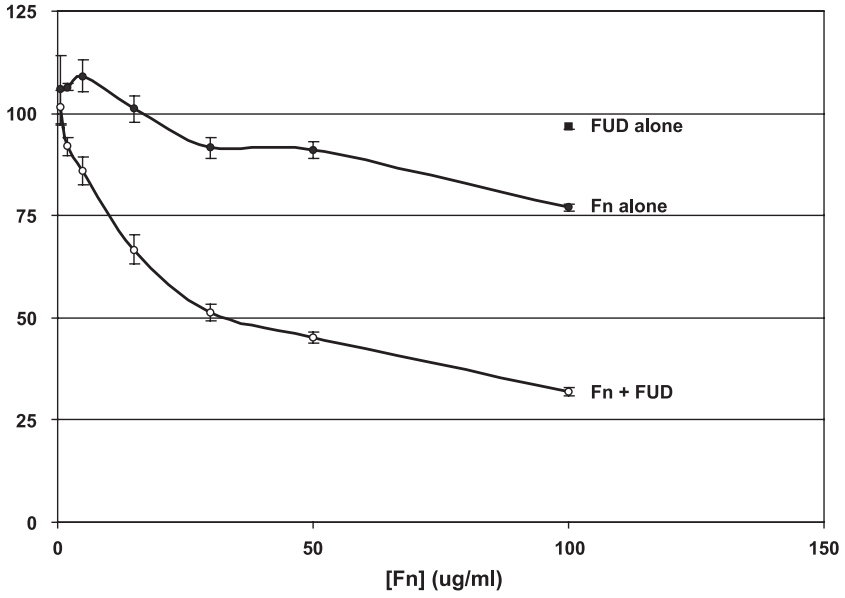

Fig. 1. Effect of FUD on the expression of the 10th type III module determined by competitive ELISA. Fn was diluted to 100 μg/ml in TBS-T and preincubated with FUD, 10 μg/ml, for 30 min at room temperature. The Fn/FUD solution was then diluted to the following Fn/FUD concentrations (μg/ml): 50:5, 30:3, 15:1.5, 5:0.5, 2:0.2, 0.5:0.05. mAbIII-10 was added at a dilution determined by ELISA to give maximal response, but still be limiting and the mixture was immediately added to microtiter wells coated with Fn (2 μg/ml). Binding of mAbIII-10 to the Fn-coated plate was quantified by ELISA and plotted as the absorbance (% of mAbIII-10 alone) versus concentration of Fn/FUD mixture (shown as Fn concentration). Fn alone is plotted as closed circles, Fn+FUD is shown as open circles. FUD alone (closed square) showed only slight inhibition. Error bars represent the standard deviation.

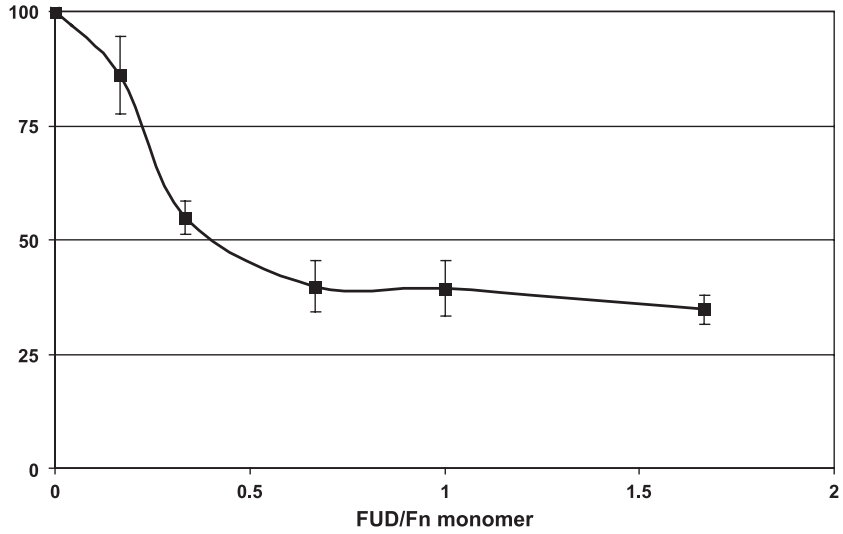

Fig. 2. Effects of increasing FUD concentrations on epitope expression. Fn, 50 μg/ml, was preincubated with FUD in TBS-T at the following molar ratios (FUD/Fn monomer): 1:6, 1:3, 2:3, 3:3, and 5:3 for 30 min at room temperature. Competitive ELISAs were done as outlined in Materials and methods. Results were normalized to Fns competitive ability. Error bars show standard error of three experiments.

In Fig. 2, we show FUD's ability to alter Fn's conformation at various stoichiometries. In the presence of increasing molar ratios of FUD, Fn exhibited an enhancement in its ability to compete with deposited Fn for mAbIII-10 binding. This competitive ability was evident at FUD/Fn monomer ratios as low as 1:6 and reached maximal levels by a 2:3 ratio.

We next determined the specificity of FUDs effect on epitope exposure of Fn. To do this, we used monoclonal antibody 9D2 which recognizes an epitope in the first type III module of Fn [37] in a competitive ELISA. The competitive ability of Fn for 9D2 was not enhanced by FUD (Fig. 3). Moreover, the slope of the curve shows that Fn in solution was able to compete at lower ratios and more completely for 9D2 binding than it was for mAbIII-10. These results show that the epitope recognized by 9D2 is fully exposed in native Fn in solution. Furthermore, the conformational changes induced by FUD have no effect on this exposure.

We have shown that FUD binds the amino-terminal 70-kDa region of Fn in a specific way independent of type III modules [33]. With this in mind, we used FnIII1-C, a

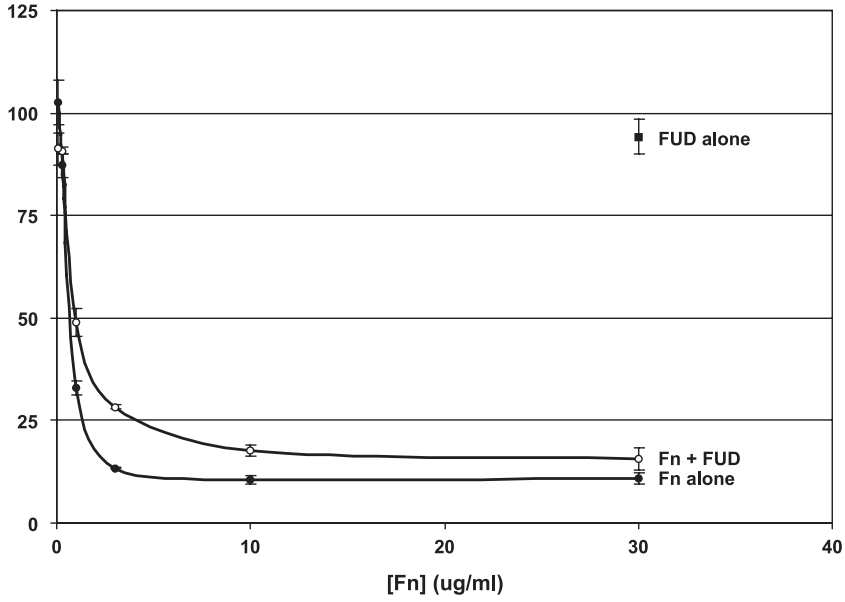

Fig. 3. Competitive ELISA antibody control. Competitive ELISAs were done as in Fig. 1 except for the use of the antibody 9D2, which recognizes an epitope on module III-1 of Fn. Results were plotted as the absorbance (% of 9D2 alone) versus concentration of Fn/FUD mixture (shown as Fn concentration) as in Fig. 1. Fn alone is plotted as closed circles, Fn+FUD is shown as open circles. FUD alone (closed squares) showed only slight inhibition. Error bars represent the standard deviation.

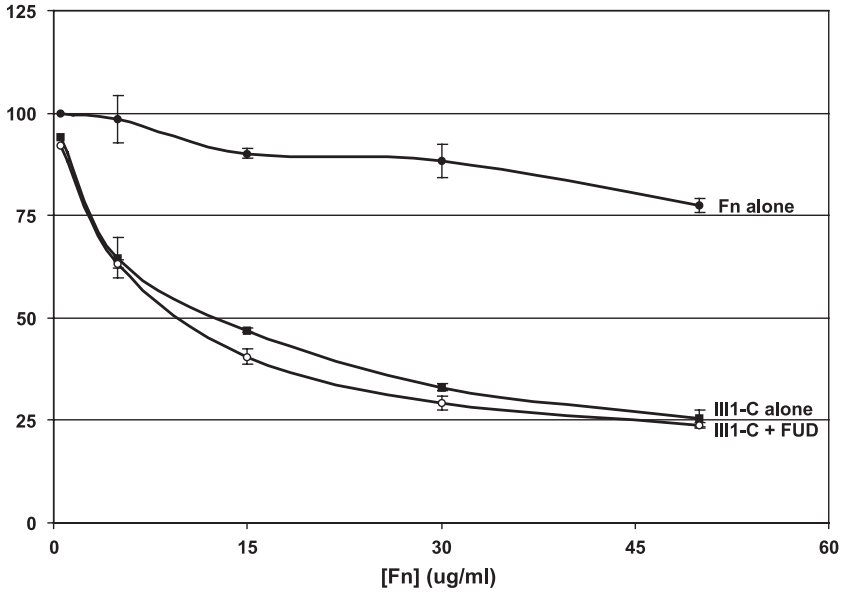

Fig. 4. Exposure of the epitope for mAbIII-10 in a Fn construct lacking the amino-terminal type I modules. Competitive ELISAs were done as in Fig. 1, except for the use of recombinant Fn fragment III1-C as the competitor. Results were plotted as the absorbance (% of mAbIII-10 alone) versus concentration of FnIII1-C:FUD mixture (shown as FnIII1-C concentrations). Fn alone is shown as closed circles, FnIII1-C is plotted as closed boxes, FnIII1-C+FUD is shown as open circles. Error bars show standard deviation.

recombinant version of Fn that lacks the 70-kDa region, in a competitive ELISA. FnIII1-C competed for mAbIII-10 binding much better than full-length Fn; and FUD did not enhance its competitive ability (Fig. 4). These findings indicate that the cell-binding region of FnIII1-C adopts, in solution, a conformation similar to full-length Fn in the presence of FUD.

4. Discussion

In this study, we show that the FUD region of prtF1 is capable of augmenting the structure of Fn in such a way that unveils the 10th type III module which contains the integrin binding RGD motif. In contrast, use of monoclonal antibody 9D2 in the competitive ELISA showed that FUD did not impact the structural conformation of all parts of Fn. This structural change reported by mAbIII-10 has many implications for both Fn and *S. pyogenes* strains that express prtF1. In this discussion, we explore these implications and compare our findings to a previously reported model of Fn's structure.

Ugarova et al. [36] pioneered use of mAbIII-10 in similar techniques as shown here to explore the potential of structural changes in Fn due to ionic strength and interactions with ECM components to impact the function of cell binding. They found that antibodies recognizing epitopes in the RGD containing 10th type III module (mAbIII-10) and, to a lesser extent, the ninth type III synergy site (mAbIII-9) were able to block cell adhesion to wells coated with Fn. They used these data to argue that the epitopes recognized by these antibodies are close to the sites that are crucial for Fn's integrin binding ability. The fact FUD enhances Fn competitive ability to bind mAbIII-10 suggests that it also makes Fn more competent to interact with integrin receptors. This feature of FUD may be beneficial for bacterial interaction with host cells and subsequent internalization; two key steps in bacterial virulence and resistance to immune response and antibacterial treatment.

FUD-induced exposure of the cell-binding region of Fn was evident at molar ratios of FUD/Fn monomer as low as 1:6, and reached maximal levels by 2:3 ratios. It would seem from these data that FUD binding with a single subunit of Fn is sufficient to induce the structural changes in a Fn dimer necessary to expose the cell-binding region. We have shown that FUD interacts with amino-terminal fragments of Fn with very high affinity [33]. This high affinity binding is likely necessary for these structural changes to take place at such low stoichiometric ratios.

The epitope for mAbIII-10 was fully unveiled in an Fn construct lacking the amino-terminal portion of Fn. This is in accord with the model proposed by Williams et al. [40] on the basis of viscosity and light scattering experiments done in collaboration with Paul Janmey and John Ferry. Despite a lack of complete primary sequence data at the time, it was proposed that Fn exists in a folded conformation where the amino-terminal part of each subunit folds back upon itself, while remaining elongated and flexible. High pH or ionic strength and collagen binding were proposed to disrupt this folding and further elongate Fn. Our data are consistent with the amino-termini of Fn subunits folding back upon the rest of the protein in such a way that occludes the cell-binding domain. Previously, Ugarova et al. [36]

showed that high ionic strength, heparin, heparan sulfate, gangliosides, and gelatin also unveil the cell-binding domain of Fn. Compared to these other substances, FUD of *S. pyogenes* prtF1 is especially potent at inducing similar conformational changes. To our knowledge, this is the first instance of a bacterially derived peptide altering Fn function by way of a conformational change. We are currently working to further understand and clarify this change and identifying the regions of FUD responsible for the effect.

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References

[1] P.R. Morrison, J.T. Edsall, S.G. Miller, Preparation of properties of serum and plasma proteins: XVIII. The separation of purified fibrinogen from fraction I of human plasma, JACS 70 (1948) 3103–3108.
[2] E. Ruoslahti, Fibronectin and its receptors, Ann. Rev. Biochem. 57 (1988) 375–413.
[3] M.K. Magnusson, D.F. Mosher, Fibronectin structure, assembly, and cardiovascular implications, Arterioscler. Thromb. Vasc. Biol. 18 (1998) 1363–1370.
[4] T.E. Petersen, K. Skorstengaard, K. Vibe-Pedersen, Primary structure of fibronectin, in: D.F. Mosher (Ed.), Fibronectin, Academic Press, New York, 1989, pp. 1–24.
[5] R.O. Hynes, Fibronectins, Springer-Verlag, New York, 1990.
[6] J.R. Potts, I.D. Campbell, Structure and function of fibronectin modules, Matrix Biol. 15 (1996) 313–320.
[7] M. Rocco, E. Infusini, M.G. Daga, L. Gogioso, C. Cuniberti, Models of fibronectin, EMBO J. 6 (1987) 2343–2349.
[8] R. Hynes, Molecular biology of fibronectin, Annu. Rev. Cell Biol. 1 (1985) 67–90.
[9] K.M. Yamada, Fibronectin domains and receptors, in: D.F. Mosher (Ed.), Fibronectin, Academic Press, New York, 1989, pp. 47–121.
[10] K. Sekiguchi, S. Hakomori, M. Funahashi, I. Matsumoto, N. Seno, Binding of fibronectin and its proteolytic fragments to glycosaminoglycans. Exposure of cryptic glycosaminoglycan-binding domains upon limited proteolysis, J. Biol. Chem. 258 (1983) 14359–14365.
[11] K.C. Ingham, S.A. Brew, S. Huff, S.V. Litvinovich, Cryptic self-association sites in type III modules of fibronectin, J. Biol. Chem. 272 (1997) 1718–1724.
[12] K. Wantanabe, H. Takahashi, Y. Habu, N. Kamiya-Kubushiro, S. Kamiya, H. Nakamura, H. Yajima, T. Ishii, T. Katayama, K. Miyazaki, F. Fukai, Interaction with heparin and matrix metalloproteinase 2 cleavage expose a cryptic anti-adhesive site of fibronectin, Biochemistry 39 (2000) 7138–7144.
[13] D. Craig, A. Krammer, K. Schulten, V. Vogel, Comparison of the early stages of forced unfolding for fibronectin type III modules, Proc. Natl. Acad. Sci. 98 (2001) 5590–5595.
[14] M. Gao, D. Craig, V. Vogel, K. Schulten, Identifying unfolding intermediates of FN-III10 by steered molecular dynamics, J. Mol. Biol. 323 (2002) 939–950.
[15] H.P. Erickson, N.A. Carrell, Fibronectin in extended and compact conformations. Electron Microscopy and Sedimentation Analysis, J. Biol. Chem. 258 (1983) 14539–14544.
[16] K.J. Johnson, H. Sage, G. Briscoe, H.P. Erickson, The compact conformation of fibronectin is determined by intramolecular ionic interactions, J. Biol. Chem. 274 (1999) 15473–15479.
[17] H.P. Erickson, Reversible unfolding of fibronectin type III and immunoglobulin domains provides the structural basis for stretch and elasticity of titin and fibronectin, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 10114–10118.
[18] R.O. Hynes, The dynamic dialogue between cell and matrices: implications of fibronectin's elasticity, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 2580–2588.
[19] A. Shaub, Unraveling the extracellular matrix, Nat. Cell Biol. 1 (1999) E173–E175.
[20] J.M. Patti, B.L. Allen, M.J. McGavin, M. Höök, MSCRAMM-mediated adherence of microorganisms to host tissues, Annu. Rev. Microbiol. 48 (1994) 585–617.
[21] A.L. Bisno, D.L. Stephens, Streptococcal infections of skin and soft tissue, N. Engl. J. Med. 334 (1996) 240–245.
[22] D. LaPenta, C. Rubens, E. Chi, P.P. Cleary, Group A streptococci efficiently invade human respiratory epithelial cells, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 12115–12119.
[23] R. Greco, L. DeMartino, G. Donnarumma, M.P. Conte, L. Seganti, P. Valenti, Invasion of cultured human cells by *Streptococcus pyogenes*, Res. Microbiol. 146 (1995) 551–560.
[24] G. Molinari, S.R. Talay, P. Valenin-Weigand, M. Rohde, G.S. Chhatwal, The fibronectin-binding protein of *Streptococcus pyogenes*, SfbI, is involved in the internalization of group A streptococci by epithelial cells, Infect. Immun. 65 (1997) 1357–1363.
[25] P.P. Cleary, D. Cue, High frequency invasion of mammalian cells by beta hemolytic *streptococci*, Sub-cell. Biochem. 33 (2000) 137–166.
[26] A. Österlund, R. Popa, T. Nikkila, A. Scheynius, L. Engstrand, Intracellular reservoir of *Streptococcus pyogenes* in vivo: a possible explanation for recurrent pharyngotonsillitis, Laryngoscope 107 (1997) 640–647.
[27] R. Neeman, N. Keller, A. Barzilai, Z. Korenman, S. Sela, Prevalence of internalization-associated gene, prtF1, among persisting group-A streptococcus strains isolated from asymptomatic carriers, Lancet 352 (1998) 1974–1997.
[28] U. Schwarz-Linek, J.M. Werner, A.R. Pickford, S. Gurusiddappa, J.H. Kim, E.S. Pilka, J.A.G. Briggs, T.S. Gough, M. Höök, I.D. Campbell, J.R. Potts, Pathogenic bacteria attach to human fibronectin through a tandem β-zipper, Nature 423 (2003) 177–181.
[29] E. Hanski, M. Caparon, Protein F, a fibronectin-binding protein, is an adhesin of the group A streptococcus *Streptococcus pyogenes*, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 6172–6176.
[30] C. Signäs, G. Raucci, K. Jösson, P.E. Lindgren, F.M. Anantharamaiah, M. Höök, M. Lindberg, Nucleotide sequence of the gene for a fibronectin-binding protein from *Staphylococcus aureus*: use of this peptide sequence in the synthesis of biologically active peptides, Proc. Natl. Acad. Sci. U. S. A. 86 (1989) 699–703.
[31] A. Sela, A. Aviv, A. Tovi, I. Burstein, M.G. Caparon, E. Hanski, Protein F: an adhesin of *streptococcus pyogenes* binds fibronectin via two distinct domains, Mol. Microbiol. 10 (1993) 1049–1055.
[32] V. Ozeri, A. Tovi, I. Burstein, S. Natanson-yaron, M.G. Caparon, K.M. Yamada, S.K. Akiyama, I. Vlodavsky, E. Hanski, A two-domain mechanism for group A *streptococcal* adherence through protein F to the extracellular matrix, EMBO J. 15 (1996) 989–998.
[33] M.G. Ensenberger, B.R. Tomasini-Johansson, J. Sottile, V. Ozeri, E. Hanski, D.F. Mosher, Specific interactions between F1 adhesin of *Streptococcus pyogenes* and N-terminal modules of fibronectin, J. Biol. Chem. 276 (2001) 35606–35613.

[34] B.R. Tomasini-Johansson, N.R. Kaufman, M.G. Ensenberger, V. Ozeri, E. Hanski, D.F. Mosher, A 49-residue peptide from adhesin F1 of *Streptococcus pyogenes* inhibits fibronectin matrix assembly, J. Biol. Chem. 276 (2001) 23430–23439.

[35] D.F. Mosher, R.B. Johnson, In vitro formation of disulfide-bonded fibronectin multimers, J. Biol. Chem. 258 (1983) 6595–6601.

[36] T.P. Ugarova, C. Zamarron, Y. Veklich, R.D. Bowditch, M.H. Ginsberg, J.W. Weisel, E.F. Plow, Conformational transitions in the cell binding domain of fibronectin, Biochemistry 34 (1995) 4457–4466.

[37] M.A. Chernousov, F.J. Fogerty, V.E. Koteliansky, D.F. Mosher, Role of the I-9 and III-1 modules of fibronectin in formation of an extracellular fibronectin matrix, J. Biol. Chem. 266 (1991) 10851–10858.

[38] D.F. Mosher, K.G. Huwiler, T.M. Misenheimer, D.S. Annis, Expression of recombinant matrix components using baculoviruses, Methods Cell Biol. 69 (2002) 69–81.

[39] S. Dufour, A. Gutman, F. Boris, N. Lamb, J.P. Thiery, A.R. Kornblihtt, Generation of full-length cDNA recombinant vectors for the transient expression of human fibronectin in mammalian cell lines, Exp. Cell Res. 193 (1991) 331–338.

[40] E.C. Williams, P.A. Janmey, J.D. Ferry, D.F. Mosher, Conformational states of fibronectin. Effects of pH, ionic strength, and collagen binding, J. Biol. Chem. 257 (1982) 14973–14978.

[41] R. Pankov, K.M. Yamada, Fibronectin at a glance, J. Cell Sci. 115 (2002) 3861–3863.