Interdomain Tilt Angle Determines Integrin-dependent Function of the Ninth and Tenth FIII Domains of Human Fibronectin*

Received for publication, June 22, 2004, and in revised form, October 7, 2004
Published, JBC Papers in Press, October 12, 2004, DOI 10.1074/jbc.M406976200

Harri Altroff‡§, Robin Schlinkert§§, Christopher F. van der Walle‡, Andrea Bernini¶¶, Iain D. Campbell§, Jörn M. Werner¶¶¶, and Helen J. Mardon$$$ From the ‡Nuffield Department of Obstetrics and Gynaecology, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU, United Kingdom and the ¶¶Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, United Kingdom

Integrins are an important family of signaling receptors that mediate diverse cellular processes. The binding of the abundant extracellular matrix ligand fibronectin to integrins αβ1 and αβ3 is known to depend upon the Arg-Gly-Asp (RGD) motif on the tenth fibronectin FIII domain. The adjacent ninth FIII domain provides a synergistic effect on RGD-mediated integrin αβ1 binding and downstream function. The precise molecular basis of this synergy remains elusive. Here we have dissected further the function of FII9 in integrin binding by analyzing the biological activity of the FII9–10 interdomain interface variants and by determining their structural and dynamic properties in solution. We demonstrate that the contribution of FII9 to both αβ1 and αβ3 binding and downstream function critically depends upon the interdomain tilt between the FII9 and FII10 domains. Our data suggest that modulation of integrin binding by FII9 may arise in part from its steric properties that determine accessibility of the RGD motif. These findings have wider implications for mechanisms of integrin-ligand binding in the physiological context.

The interaction of the abundant extracellular matrix molecule fibronectin with the integrin family of cell surface receptors mediates wide-ranging biological processes, including cell migration, invasion, and angiogenesis. Integrin-fibronectin interactions thus have a fundamental role in tissue development and homeostasis, and integrin receptor agonism and antagonism are implicated in the etiology of a number of diseases such as cancer and inflammatory disorders. However, the precise molecular bases of integrin-fibronectin interactions required for downstream biological function are not understood.

Integrins are heterodimeric cell surface receptors comprising one α and one β subunit. So far, 18 α and 8 β subunits have been identified, but the potential to form functional dimers is restricted. Some integrin subunits, notably αv and β3, are more promiscuous than others, such as α5, in the selection of subunits with which they can dimerize. Similarly, some integrins, such as αβ3, can bind to more than a dozen different ligands including fibronectin, whereas the ligand repertoire of others, such as αβ1, is much more limited.

Fibronectin is a large dimeric glycoprotein (~220 kDa) and is almost exclusively composed of repeating units falling into three structurally distinct categories, named type I (FII1), type II (FII1), and type III (FIIII) domains. The central portion of the molecule contains a pair of FIIII domains, designated FII9 and FII10, which constitute the best studied and most versatile integrin-binding region. The tripeptide adhesion motif Arg-Gly-Asp (RGD) resides on a surface loop in FII10 and is the essential recognition site for at least 11 integrins. The adjacent FII9 domain provides synergistic enhancement of binding to and signaling through several RGD-dependent integrins, including αβ1 (1–5) and αβ3 (6, 7). In contrast, αβ3 is generally believed not to require this synergistic effect (5, 7).

Numerous studies of the interactions of the FII9–10 pair with integrins have demonstrated the functional importance of specific synergistic residues or sequences, notably the PHSRN motif, within FII9 (2–4, 6–9). The structural basis for the synergy phenomenon, however, is elusive, and several different mechanisms have been proposed. The most obvious is a direct contribution from FII9 to integrin binding (8, 10). Solution x-ray scattering and mutagenesis studies performed recently suggest the existence of direct interactions between FII9 and the integrin α subunit (10). However, in a second study, electron micrographs did not reveal significant FII9-integrin contact (11). Recent data from this laboratory suggest that the FII9 domain can modulate binding via less specific, conformational effects affecting global domain stability (2, 3). In addition, partial structural uncoupling of FII9 and FII10 by the extension of the linker at the FII9–10 interface leads to loss of synergistic function (12), indicating a requirement for adequate interdomain contact for receptor binding.

Although flexibility of the FII9–10 linkage has been implicated in large scale conformational changes in native fibronectin (13), there is no direct evidence to support this, and the potential biological consequences of such structural changes are not known. Here we explore the possibility that modulation of the interdomain tilt between FII9 and FII10 may affect the domain pair’s integrin binding and downstream biological activity. Our aim is to gain further structural insight into the mechanism of integrin recognition by fibronectin.

EXPERIMENTAL PROCEDURES

Construction and Expression of FIIII Variants—Wild-type FII9–10 and FII10 clones were obtained by inserting the respective DNA frag-
ments, amplified from the pGEX2T-FII9–10 construct (14) and
digested with NheI/HindIII, into the corresponding sites in the pRSET-A
vector (Invitrogen). Creation of the FII9–10 construct incorporating a
Leu1408→Pro mutation (designated here as FII9-10) has been de-
scribed previously (15). Further amino acid substitutions, Ala1340/
Val1442→Cys/Cys, were introduced into the latter construct according
to the QuikChange™ protocol (Stratagene), resulting in mutant
FII9-10-CC. All FII variants were expressed as His tag proteins in
*Escherichia coli* strain BL21(DE3)pLysS (Promega) and purified using
Ni²⁺-nitrilotriacetic acid Superflow resin (Qiagen). Isotopically labeled
protein was produced using modified M9 medium containing 5 µliter
¹⁵NH₄Cl and/or 2 µliter uniformly labeled [¹³C]glucose (CK Gas Prod-
ucts). Purity of the proteins was assessed by SDS-PAGE and mass
spectrometry.

The single disulfide bond present in the oxidized form of mutant
FII9-10-CC was reduced either by adding dithiothreitol at 100–400
times molar excess (for NεH and equilibrium unfolding experiments) or
by alkylation with iodoacetamide (for cell association and solid-phase
assays). The oxidation state of mutant FII9-10-CC was confirmed by
mass spectrometry.

Enzyme-linked Immunosorbent Assays—Integrin αβ, used in solid-
phase binding assays was purified from human placenta as specified
elsewhere (3). Purification of αβ was performed in a similar manner
using a Ni²⁺-column (HiLoad 9/10) raised against the αβ heterodimer.
Binding of soluble FII constructs to immobilized integrin was per-
formed in the presence of divalent cations Mn²⁺, Mg²⁺, and Ca²⁺ as
described earlier (3), except that horseradish peroxidase-conjugated
anti-HisG antibody (Invitrogen) at 0.4 µg/ml was used for detection,
followed by color development with the Sigma Fast™ tablet set. Data
were analyzed by nonlinear regression as described (3). Assay results are
expressed as the percentage of the wild-type values ±S.E. (dose-response
graphs) or the means ±S.E. (one-letter code). The dotted line designates
disulfide bond present in oxidizing conditions. The synergistic PHSRN sequence and the RGD
motif are indicated in yellow and blue, respectively.

FIG. 1. Ribbon diagram of the FII9–10 domain pair. Atomic
coordinates were obtained from the Protein Data Bank (43) (Protein
Data Bank code 1FPF) and imaged using RasTop (available on the
World Wide Web at www.scripps.edu/ncihg寮). Residues substituted in
this study, shown in their putative mutant conformation as rendered by
Swiss PDB Viewer (available on the World Wide Web at spdbv.nih.gov),
are displayed in ball-and-stick format and labeled accordingly using one-letter code. The
boxed domain pair is rendered in ribbon form in yellow and blue.

NMR Sample Preparation—All NMR samples were prepared from
freeze-dried ¹⁵N-labeled protein in a 50 mM sodium acetate buffer in
95/5% H₂O/D₂O. The pH was adjusted to 4.8 by the addition of HCl. 4% polycrylamide gels with 3% cross-linking were produced using modified M9 medium containing 5 µliter
¹⁵NH₄Cl and/or 2 µliter uniformly labeled [¹³C]glucose (CK Gas Prod-
ucts).

NMR Experiments—All NMR experiments were performed at 25, 30,
or 35 °C on spectrometers operating at ¹H frequencies of 600 and 750
MHz with triple-resonance triaxial gradient probes. Experiments used
gradient-enhanced coherence selection (20) and water flip-back (21)
wherever possible. For backbone assignment of FII9-10, three-dimen-
sional ¹⁵N-edited NOEY-HSQC (22) and TOCSY-HSQC (23) spectra
with mixing times of 125 and 29.0 ms, respectively, and a TOCSY field
strength of 11,900 Hz were recorded on a 1 mm sample at 600 MHz,
and as the three-dimensional triple-resonance experiments HNCA (24),
HNOCA (25), and CBCACONH (26) at 750 MHz. H₁ N, HN, and NH
resonances were assigned using a combination of cross-peaks in the
three-dimensional ¹⁵N-edited TOCSY and NOEY-HSQC spectra be-
 tween H₁ N, HN, and NH to NH resonances as well as through bond
correlations between the C(i) and C(i-1) and C(i) observed in the HNCA
and HNCOCA. Characteristic C(i)/C(i-1) shifts in the CBCACONH aided the
identification of amino acid residues (27). Initial assignments of NH and ¹⁵N
resonances of the FII9-10-CC mutant were derived from FII9-10, and
these were confirmed through H₂ N, HN, to NH, and NH to NH cross-peaks
in the three-dimensional ¹⁵N-edited NOEY-HSQC.

Heteronuclear [¹H]¹⁵N NOE spectra were acquired on 1 mm samples
at 30 °C and 600 MHz with acquisition times of 40.4 ms for ¹⁵N and 82
ms for ¹H. ¹H saturation in the NOE experiment was effected by means of
a train of 225° flip-angle pulses at 10-ms intervals for 4.5 s. ¹⁵N-1³C,T,
¹H-¹⁵N, and 1³C-1³C, one-letter code HNCOCA (24), and CBCACONH (26) at 750 MHz. H, HN, and NH resonances were assigned using a combination of cross-peaks in the
three-dimensional ¹⁵N-edited TOCSY and NOEY-HSQC spectra be-
tween H, HN, and NH to NH resonances as well as through bond
correlations between the C(i) and C(i-1) and C(i) observed in the HNCA
and HNCOCA. Characteristic C(i)/C(i-1) shifts in the CBCACONH aided the
identification of amino acid residues (27). Initial assignments of NH and ¹⁵N
resonances of the FII9-10-CC mutant were derived from FII9-10, and
these were confirmed through H₂ N, HN, to NH, and NH to NH cross-peaks
in the three-dimensional ¹⁵N-edited NOEY-HSQC.

Heteronuclear [¹H]¹⁵N NOE spectra were acquired on 1 mm samples
at 30 °C and 600 MHz with acquisition times of 40.4 ms for ¹⁵N and 82
ms for ¹H. ¹H saturation in the NOE experiment was effected by means of
a train of 225° flip-angle pulses at 10-ms intervals for 4.5 s. ¹⁵N-1³C,T,
Interdomain Tilt Angle Determines Fibronectin Function

CCox) and in the reduced form (FIII9'10-CCred). All tested proteins, except for FIII9'10-CCox, follow a two-state unfolding mechanism characteristic of native FIII9–10 (3,36) and have very similar unfolding parameters for the FIII10 domain, whose conformational stability is known to be largely independent of the presence of FIII9 (3) (Fig. 2a, inset, and Table I). Analysis of the effect of the interface mutations on the unfolding of FIII9, however, reveals that mutant FIII9'10-CC shows an increased stability both in its reduced and oxidized forms (Fig. 2). We note that the moderate stability increase seen with FIII9'10-CCred may partly arise from an incomplete reduction of the interdomain disulfide bridge by dithiothreitol. The observed stability-promoting effect is, however, greatly enhanced in FIII9 10-CCox such that unfolding of FIII9 can no longer be separated from that of FIII10, and the two domains follow a single transition curve (Fig. 2a, inset). The values for FIII9 stability in the variants, expressed as the GdnHCl concentration leading to 50% domain unfolding ([GdnHCl]), are 2.33, 3.12, and 4.43 M for FIII9', FIII9'10-CCred, and FIII9'10-CCox, respectively (Fig. 2b and Table I). These findings indicate that the unfolding process is sensitive to amino acid changes in the interface region between FIII9 and FIII10 and provide further support to the notion that the interdomain contact surface within FIII9–10 is an important determinant of the thermodynamic properties of FIII9 (36).

Interdomain Disulfide Linkage Abolishes the Synergistic Cell Adhesion Activity of FIII9–10 and Reduces Its Affinity for Integrins a, b/ and a, b3—The interface variants were next subjected to assays designed to quantify the impact of interdomain perturbations on function. Cell adhesion assays were performed with immobilized FIII9–10 proteins and BHK fibroblasts, which recognize fibronectin mainly via integrins a, b/ and a, b3 (12). Integrin a, b/ accounts for most of the adhesion potential of BHK cells and is a major synergy-dependent receptor for fibronectin, whereas a, b3 has been shown to bind the single FIII10 domain with high affinity and does not require FIII9 for full activity (5,7). The functional activity of mutant FIII9'10-CCox was drastically reduced compared with FIII9'10 and was similar to that achieved with the single FIII10 domain (Fig. 3, a and b). This was the case both for cell attachment (Fig. 3a), a primary event of establishing integrin-dependent contact with the ligand, and the ensuing cell spreading (Fig. 3b), driven by extensive signaling and reorganization of the cytoskeletal architecture. In contrast, the adhesion-promoting ability of FIII9'10-CCred was indistinguishable from that of native FIII9–10 or FIII9'10 (Fig. 3, a and b).

We further sought to complement our cell-based studies with solid-phase receptor binding assays. Dose-response data from experiments on the FIII9'10-CC interface mutant and purified integrins a, b/ and a, b3 (Fig. 3, c–f) show that the disulfide-linked FIII9'10-CCox loses the synergistic activity of its parent protein FIII9'10 for binding to a, b3. It exhibits effectively the same receptor affinity as the isolated FIII10 domain and has an apparent Kd value ~15-fold higher than that of FIII9'10 (Fig. 3, c and d). In contrast, FIII9'10-CCred has a substantially higher affinity for a, b3, although it does not reach the level of binding achieved by FIII9'10 (Fig. 3, c and d). The FIII9'10-CCox variant also exhibits a markedly reduced affinity for integrin a, b3, as compared with FIII9'10 (the apparent Kd is ~5-fold higher), whereas both FIII9'10-CCred and the single FIII10 domain bind a, b3 with an affinity similar to that of FIII9'10 (Fig. 3, e and f). These solid-phase observations are thus in general agreement with the cell adhesion data.

Our findings show that improved conformational stability of FIII9, as seen with mutant FIII9'10-CC, does not necessarily correlate with its synergistic biological activity. This prompted us to perform a detailed analysis of the structural basis of the loss of function of the disulfide-linked FIII9'10-CCox.

The Interdomain Disulfide Bridge Reduces the Average Tilt Angle between FIII9 and FIII10—The structural integrity of FIII9'10 and FIII9'10-CC was confirmed by solution state NMR. The 1H, 15N backbone resonances of the FIII9'10-CC mutant were characteristic of folded molecules and showed only small chemical shift changes with respect to the FIII9'10 variant (data not shown). Local and global changes in structure and dynamics were further determined using chemical shift perturbation, RDCs, and heteronuclear relaxation measurements.

The chemical shift differences between FIII9'10 and FIII9'10-CC in the oxidized and reduced state are shown in Fig. 4. The only significant perturbations in both FIII9'10-CCox and FIII9'10-CCred with respect to FIII9'10 are restricted to the vicinity of the mutated residues (Fig. 4). This indicates that neither the introduction of this disulfide bond nor its reduction cause a significant structural change in the backbone structure of individual domains. This was expected because the disulfide...
bridge was designed to satisfy the distance and conformational constraints on the basis of crystal structure coordinates (32).

Changes in the orientations of the NH bond vectors of FIII9–10 and the FIII9–10-CC variants were determined from the measurements of RDCs of NH bond vectors in two different alignment media. The results for the polyacrylamide medium are shown in Table II and illustrated in Fig. 5. The bicelle medium yields similar data (not shown). RDCs are very sensitive to angular reorientations of a particular bond or molecular fragment with respect to the molecular frame and are therefore

| FIII9–10 variant | Domain | [GdnHCl]_50 | ΔG_H2O | m |
|------------------|--------|-------------|--------|---|
| FIII9–10         | FIII9  | 1.28        | 3.38   | 4.02 |
| FIII9'10         | FIII10 | 5.01        | 8.28   | 1.77 |
| FIII9'10-CC_red  | FIII9  | 2.33        | 6.16   | 2.30 |
|                 | FIII10 | 5.01        | 8.28   | 1.53 |
| FIII9'10-CC_red  | FIII9–10| 4.99      | 8.25   | 1.61 |
|                 |        | 4.43        | 11.71  | 1.41 |

*a* Due to potential errors associated with determining the individual slope (m) values for the linear transition region (16), the values of ΔG(H2O) were calculated from the average m values of 2.64 and 1.65 kcal mol\(^{-1}\) M\(^{-1}\) for FIII9 and FIII10, respectively.

*b* Calculated from the average m value for FIII9.

**FIG. 3.** Effect of disulfide linkage on the biological activity of FIII9–10. 

*a*, attachment of BHK cells on surface-immobilized FIII9–10 variants and a single FIII10 domain. Results are expressed relative to the maximum level of attachment attained at higher concentrations of FIII9'10 (not shown). 

*b*, assessment of the ability of the FIII9–10 variants to support BHK cell spreading. 

*c* and *e*, normalized dose-response data for solid-phase binding of FIII proteins to surface-bound integrin α5β1 (*c*) or αvβ3 (*e*). Open squares, FIII9–10; filled squares, FIII9'10; blue circles, FIII9'10-CC_red; red circles, FIII9'10-CC_red; open triangles, FIII10. 

*d* and *f*, apparent K_d values derived from the curves in *c* and *e*, for binding of FIII9'10, FIII9'10-CC_red, FIII9'10-CC_red, and FIII10 (left to right) to integrin α5β1 (*d*) or αvβ3 (*f*).
The analysis of the interdomain orientations (Table II) shows that the time-averaged interdomain tilt angle for FIII9–10 is 28 ± 1°, whereas FIII9–10-CC_{ox} shows a reduced tilt angle of 5 ± 1°. The tilt angle in FIII9–10-CC_{red} is restored to 21 ± 1°. The values of the twist angles obtained for the three constructs range from 340 ± 5° to 355 ± 6° and are experimentally almost indistinguishable. These measurements thus indicate that the breaking and formation of the interdomain disulfide bond in FIII9–10-CC is associated with a significant change in the interdomain tilt angle but not interdomain twist. These observations correlate well with the data obtained from cell adhesion and integrin binding assays showing restoration of function for reduced FIII9–10-CC.

The Synergistic PHSRN Loop in FIII9 Is Relatively Rigid—Interdomain motion as well as modulation of the RGD loop mobility has been implicated in integrin binding and activation of FIII9. When the FIII9–10 fragment of fibronectin (32, 36). We therefore studied the dynamics of FIII9–10, FIII9–10-CC_{ox}, and FIII9–10-CC_{red} by 15N relaxation. Local internal dynamics of the backbone NH bond vectors were derived from the [1H]15N NOE (Fig. 6). Values of the [1H]15N NOE of a backbone NH below its
errors in the FIII9 of the 1 production of the disulfide bond. The RGD loop and the synergy site of the variants. This indicates that both proteins have a similar degree of interdomain motion.

We found that the loop containing the PHSRN synergy site in FIII9 is rigid compared with the RGD loop and other extended loop regions. The local dynamics of the backbone NH vectors are very similar in FIII9 and the oxidized and reduced form of FIII9-CC (Fig. 6). In particular, this applies to the RGD loop and the synergy site of the variants. This indicates that the local dynamics of the individual domains on a subnanosecond time scale is virtually unaffected by the introduction of the disulfide bond.

The Interdomain Dynamics of the FIII9 Variants Are Similar—Comparison of experimentally determined rotational diffusion tensors and the calculated diffusion tensor of a rigid model provides information on interdomain dynamics. The diffusion tensors of FIII9 and FIII9-CCox were obtained from an analysis of the T1 and T2 relaxation times (Fig. 7). The errors in the FIII9-CCred data were larger, probably due to the presence of dithiothreitol, so that determination of a diffusion tensor was considered unreliable. In FIII9 the isotropic correlation times, determined using TENSOR (see “Experimental Procedures”), were 11.9 ns for FIII9 and 12.8 ns for FIII10, and in FIII9-CCox they were 12.4 and 13.6 ns, respectively. For individual modules in both FIII9 and FIII9-CCox a prolate diffusion tensor with an axial ratio (Dshort/Dlong) of ~1.8 ± 0.3 yielded an adequate description of the data, indicating that in solution both molecules are cigar-shaped. The small difference in correlation times of the individual domains in each protein and the fact that the axial ratio of the long axis to the short axis (Dshort/Dlong) in both FIII9 and FIII9-CCox is smaller than that expected from a hydrated rigid structure suggest that both proteins have a similar degree of interdomain motion.

We conclude from these structural analyses that the primary effect of the introduction of the disulfide bond between residues Cys1340 and Cys1442 at the interdomain interface of FIII9 is a reduction in the interdomain tilt angle. Structural and dynamic changes are localized to the vicinity of the mutated residues. The average interdomain orientation in FIII9 in solution shows a greater tilt angle than the x-ray structure, with the RGD site more prominently exposed by the tilt. In contrast, the tilt angle in FIII9-CCox is significantly reduced. All FIII9-CCox variant proteins appear to have a similar degree of interdomain flexibility.

DISCUSSION

The structure of integrin α5β3 complexed with an Arg-Gly-Asp peptide (40) provided groundbreaking insight into how integrins interact with specific active motifs within their ligands, but the precise molecular mechanism by which large extracellular matrix proteins bind RGD-dependent integrins remains unclear. A major issue is the nature of the synergistic effect of FII9 on RGD-mediated binding of fibronectin to integrin α5β3. Here we provide evidence for a further structural determinant for fibronectin-integrin interactions by demonstrating that the FII9-FIII10 interdomain tilt has a profound effect upon integrin binding and function.

There are currently a number of different and conflicting models to explain how the synergy between FII9 and FIII10 is achieved. Several lines of evidence suggest that a synergy site in FII9, containing the residues PHSRN, exerts an effect by making direct contact with integrins such as α5β3 (4, 8, 9, 12). These include observations that the distance between the RGD...
loop and the PHSRN loop and the degree of interdomain coupling are important for optimal integrin activity (12, 36). Point mutations in the synergy site have been shown to be important for both integrin binding and downstream function (2–4, 6, 7, 9). However, some of the effects of these mutants can be reversed by the addition of contiguous FIII domains or by stability-conferring mutations in FIII9 (2, 3). This suggests that FIII9 provides long range stabilization of critical integrin-binding motifs in the FIII9–10 pair and that the presence of correctly folded FIII9 is required to ensure proper orientation of the interdomain interface disulfide bond. This strengthens the notion that the effect of the introduction of the interdomain interface disulfide bond creates a fixed orientation of FIII9. One explanation of our data is that the introduction of the interdomain interface disulfide bond creates a fixed orientation for the FIII9 synergy site with respect to the RGD loop in FIII10 that is not readily accommodated by the surface of the αv subunit. The observed low flexibility of the loop in FIII9 carrying the PHSRN synergy site would further complicate any conformational adaptation. This would be consistent with a direct interaction of the synergy site residues with the β-propeller domain of αv (10). However an alternative explanation is that the RGD loop is more accessible in some domain orientations than others, although it is observed to remain mobile in all of the variants analyzed. According to this scenario, the reduction in the tilt between FIII9 and FIII10 introduces a steric effect by FIII9 that precludes sufficient interaction of the RGD loop with its binding pocket at the integrin αvβ3 surface. Binding of the disulfide-linked FIII9–10-CC6 to integrin αvβ3 is reduced to a level similar to that achieved by FIII10, suggesting that the less tilted FIII9 domain does not block binding of the RGD motif to αvβ3, but restricts any synergistic steering effect on integrin recognition. This may in part explain how integrin specificity can arise in response to changes in interdomain orientation. One possibility is that the RGD binding pocket on αvβ3 is less deep and more accessible to the ligand than the corresponding interface on αvβ5, thus making αvβ3 more tolerant of steric interference by the reoriented FIII9. This scenario does not exclude the use of motifs in FIII9 for direct integrin binding but can readily explain the reduced binding of FIII9–10-CC6 to a synergy-independent integrin such as αvβ3.

Whatever the mechanism, we predict that such changes in...
the interdomain tilt between FIII9 and FIII10 can be achieved in situ because of the interdomain flexibility, which is inferred to be unusually high for this domain pair (32, 41). Previous structural data suggest that the integrin headpiece comprising an α subunit β-propeller and a β subunit I-like domain does not undergo large conformational changes upon binding to fibronectin-derived ligands (10, 11, 40). Flexibility of the FIII9–10 pair, as revealed here and inferred from previous studies on human (11, 36) and mouse (39) FIII9–10, may thus allow conformational changes that are required to accommodate binding to the integrin headpiece.

In summary, we have demonstrated that a change in fibronectin domain tilt can act as a switch between high and low integrin binding activity. Such changes in the interdomain tilt angle may be important physiologically in accommodating structural differences between the low activity, compact form of soluble plasma fibronectin and the high activity, fibrillar form of matrix fibronectin (13, 42). In addition, changes in the FIII9–10 interdomain tilt may occur in situ in response to binding of other extracellular matrix components at proximal and/or distal sites on the fibronectin molecule. We therefore propose that modulation of interdomain tilt provides an additional mechanism for fine-tuning fibronectin-integrin interactions.

REFERENCES

1. Obara, M., and Yoshizato, K. (1995) Exp. Cell Res. 216, 273–276
2. Altroff, H., Choulier, L., and Mardon, H. J. (2003) J. Biol. Chem. 278, 491–497
3. Altroff, H., van der Walle, C. F., Asselin, J., Fairless, R., Campbell, I. D., and Mardon, H. J. (2001) J. Biol. Chem. 276, 38885–38892
4. Redick, S. D., Settles, D. L., Briscoe, G., and Erickson, H. P. (2000) J. Cell Biol. 149, 521–527
5. Akiyama, S. K., Aota, S., and Yamada, K. M. (1995) Cell Adhes. Commun. 3, 13–25
6. Kauf, A. C., Hough, S. M., and Bowditch, R. D. (2001) Biochemistry 40, 9159–9166
7. Bowditch, R. D., Hariharan, M., Teminna, K. F., Smith, J. W., Yamada, K. M., Getzoff, E. D., and Ginsberg, M. H. (1994) J. Biol. Chem. 269, 10856–10863
8. Mould, A. P., Askari, J. A., Aota, S., Yamada, K. M., Irie, A., Takada, Y., Mardon, H. J., and Humphries, M. J. (1997) J. Biol. Chem. 272, 17283–17292
9. Aota, S., Nomizu, M., and Yamada, K. M. (1994) J. Biol. Chem. 269, 24756–24761
10. Mould, A. P., Symonds, E. J., Buckley, P. A., Grossmann, J. G., McEwan, P. A., Barton, S. J., Askari, J. A., Craiz, S. E., Bella, J., and Humphries, M. J. (2003) J. Biol. Chem. 278, 39993–39999
11. Takagi, J., Strokovich, K., Springer, T. A., and Walz, T. (2005) EMBO J. 22, 4607–4615
12. Grant, R. P., Spitzfaden, C., Altroff, H., Campbell, I. D., and Mardon, H. J. (1997) *J. Biol. Chem.* **272**, 6159–6166.
13. Johnson, K. J., Sage, H., Briscoe, G., and Erickson, H. P. (1999) *J. Biol. Chem.* **274**, 15473–15479.
14. Mardon, H. J., and Grant, K. E. (1994) *FEBS Lett.* **340**, 197–201.
15. van der Walle, C. F., Altroff, H., and Mardon, H. J. (2002) *Protein Eng.* **15**, 1021–1024.
16. Pace, C. N., and Scholtz, J. M. (1997) in *Protein Structure: A Practical Approach* (Creighton, T. E., ed.) pp. 299–321, IRL Press, Oxford, UK.
17. Sass, H. J., Musco, G., Stahl, S. J., Wingfield, P. T., and Grzesiek, S. (2000) *J. Biomol. NMR* **18**, 303–309.
18. Ishii, Y., Markus, M. A., and Tycko, R. (2001) *J. Biomol. NMR* **18**, 141–151.
19. Chou, J. J., Gaemers, S., Howder, B., Louis, J. M., and Bax, A. (2001) *J. Biomol. NMR* **21**, 377–382.
20. Kay, L. E., Keifer, P., and Saarinen, T. (1992) *J. Am. Chem. Soc.* **114**, 10663–10665.
21. Grzesiek, S., and Bax, A. (1993) *J. Am. Chem. Soc.* **115**, 12593–12594.
22. Kay, L. E., Marion, D., and Bax, A. (1989) *J. Magn. Reson.* **84**, 72–84.
23. Driscoll, P. C., Clore, G. M., Marion, D., Wingfield, P. T., and Gronenborn, A. M. (1990) *Biochemistry* **29**, 3542–3556.
24. Kay, L. E., Ikura, M., Tschudin, R., and Bax, A. (1990) *J. Magn. Reson.* **89**, 496–514.
25. Grzesiek, S., and Bax, A. (1992) *J. Magn. Reson.* **96**, 432–440.
26. Grzesiek, S., and Bax, A. (1992) *J. Am. Chem. Soc.* **114**, 6291–6293.
27. Grzesiek, S., and Bax, A. (1993) *J. Biomol. NMR* **3**, 185–204.
28. Farrow, N. A., Zhang, O., Forman-Kay, J. D., and Kay, L. E. (1994) *J. Biomol. NMR* **4**, 727–734.
29. Ottiger, M., Delaglio, F., and Bax, A. (1998) *J. Magn. Reson.* **131**, 373–378.
30. Johnson, B. A., and Blevins, R. A. (1994) *J. Biomol. NMR* **4**, 603–614.
31. Desmet, P., Huo, J. C., Marion, D., and Blackledge, M. (2001) *J. Biomol. NMR* **20**, 225–231.
32. Leahy, D. J., Aukhil, I., and Erickson, H. P. (1996) *Cell* **84**, 155–164.
33. Bork, P., Downing, A. K., Kieffer, B., and Campbell, I. D. (1996) *Q. Rev. Biophys.* **29**, 119–167.
34. Werner, J. M., Campbell, I. D., and Downing, A. K. (2001) *Methods Mol. Biol.* **173**, 285–300.
35. Desmet, P., Huo, J. C., Blackledge, M., and Marion, D. (2000) *J. Biomol. NMR* **16**, 23–28.
36. Spitzfaden, C., Grant, R. P., Mardon, H. J., and Campbell, I. D. (1997) *J. Mol. Biol.* **265**, 565–579.
37. Bax, A., Kontaxis, G., and Tjandra, N. (2001) *Methods Enzymol.* **339**, 127–174.
38. Main, A. L., Harvey, T. S., Baron, M., Boyd, J., and Campbell, I. D. (1992) *Cell* **71**, 671–678.
39. Copie, V., Tomita, Y., Akiyama, S. K., Aota, S., Yamada, K. M., Venable, R. M., Pastor, R. W., Krueger, S., and Torchia, D. A. (1998) *J. Mol. Biol.* **277**, 663–682.
40. Xiong, J. P., Stehle, T., Zhang, R., Joachimiak, A., Frech, M., Goodman, S. L., and Arnaout, M. A. (2002) *Science* **296**, 151–155.
41. Sharma, A., Askari, J. A., Humphries, M. J., Jones, E. Y., and Stuart, D. I. (1999) *EMBO J.* **18**, 1468–1479.
42. Ugurova, T. P., Zamarro, C., Veklich, Y., Bowditch, R. D., Ginsberg, M. H., Weisel, J. W., and Plow, E. F. (1995) *Biochemistry* **34**, 4457–4466.
43. Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., Shindyalov, I. N., and Bourne, P. E. (2000) *Nucleic Acids Res.* **28**, 235–242.
44. Lipari, G., and Szabo, A. (1982) *J. Am. Chem. Soc.* **104**, 4546–4559.