Vol. 217, No. 2, 1995 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
December 14, 1995 Pages 466-474

DIRECT BINDING OF F ACTIN TO THE CYTOPLASMIC DOMAIN OF THE α2 INTEGRIN CHAIN IN VITRO

J. David Kieffer*, George Plopper+, Donald E. Ingber+, John H. Hartwig#, and Thomas S. Kupper* ^

* Harvard Skin Disease Research Center, Division of Dermatology, Brigham and Women's Hospital, Boston, MA 02115

+ Departments of Surgery and Pathology, Children's Hospital and Harvard Medical School, Boston, MA 02115

Department of Anatomy and Cellular Biology, Harvard Medical School, Boston, MA 02115

Received November 3, 1995

Summary: The transmembrane integrins have been shown to interact with the cytoskeleton via noncovalent binding between cytoplasmic domains (CDs) of integrin β chains and various actin binding proteins within the focal adhesion complex. Direct or indirect integrin α chain CD binding to the actin cytoskeleton has not been reported. We show here that actin, as an abundant constituent of focal adhesion complex proteins isolated from fibroblasts, binds strongly and specifically to α2 CD, but not to α1 CD peptide. Similar specific binding to α2 CD peptide was seen for highly purified F actin, free of putative actin-binding proteins. The bound complex of actin and peptide was visualized directly by coprecipitation, and actin binding was abrogated by removal of a five amino acid sequence from the α2 CD peptide. Our findings may explain the earlier observation that, while integrins α2β1 and α1β1 both bind to collagen, only α2β1 can mediate contraction of extracellular collagen matrices.

Interactions between integrins and cytoskeletal filaments are important for processes including tissue remodeling, embryogenesis, and mechanosensation (1-3). Current models (4-8) depict interactions between integrins and the cytoskeleton via binding of the CDs of integrin β chains to various actin binding proteins within the focal adhesion complex (FAC). Direct involvement of integrin α chain CDs in such linkages has not been reported, although recent evidence suggests that α chain CDs can modify the localization of integrins into FACs by masking sites on β chain CDs (9). However, we have observed that while the extracellular domains of the integrins α2β1 and α1β1 bind to similar sequences on type I collagen with comparable affinity, α2β1

^ To whom correspondence should be addressed at Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115. Fax: (617) 278-0305.

0006-291X/95 $12.00

can mediate collagen gel contraction while $\alpha 1\beta 1$ does not, indicating a role of the $\alpha 2$ CD in the transmission of contractile forces (10,11). In accordance with this concept, cells expressing a chimeric integrin with the extracellular region of $\alpha 2$ and the CD of $\alpha 4$ failed to contract collagen gels (12). In this study, we have explored the possibility that α chain CDs directly interact with elements of the FAC by comparing the binding of FAC proteins to $\alpha 1$ CD and $\alpha 2$ CD peptides. We have identified a prominent FAC protein specifically binding to $\alpha 2$ CD, but not $\alpha 1$ CD, peptide as actin. In addition, we have documented similar specific binding to $\alpha 2$ CD peptide for purified F actin, free of putative actin-binding proteins. These are the first findings to suggest a model in which 1) integrin α chain CDs, in addition to β chain CDs, are directly involved in integrin-cytoskeleton linkage, and 2) actin is linked directly to an integrin CD, rather than via an actin binding protein.

MATERIALS AND METHODS

Cell Culture. Human foreskin fibroblasts for the study of cellular FACs were isolated from explant cultures. The cells were grown in DMEM supplemented with 10% fetal calf serum and antibiotics. Approximately 10^8 fibroblasts were metabolically labeled with ^{35}S -methionine/cysteine (ICN), by standard methods (13).

Focal Adhesion Complexes. FACs were isolated using magnetic microbeads coated with type I collagen, as described (14). FAC proteins were extracted from the washed microbeads by overnight incubation at 4°C in 1% Triton X-100 and 125 μg/ml aprotinin and leupeptin in low salt buffer (50mM NaCl, 50mM Tris acetate, 10mM EGTA, 2mM $MgCl_2$, pH 7.6). The microbeads were then removed by centrifugation. The extracts were subjected to affinity chromatography as described below.

Actin. Purified unlabeled rabbit skeletal muscle actin was prepared as reported (15). In most of our experiments with this material (Figs. 2a, 2c, 3, and 4), the actin, initially in monomeric form (G-actin), was diluted 10-20 fold into the low salt-Triton buffer described above, shortly before application to the affinity columns at a final concentration of 10 μM actin. The ionic conditions used strongly favor polymerization of actin (16). Thus, we were confident that F actin was responsible for the observed binding. To confirm this point, we compared the binding to $\alpha 2$ CD peptide columns of actin assumed polymerized by ionic conditions and explicitly polymerized actin under three conditions of stabilization and filament length (Fig. 2b). One sample of actin was diluted directly into low salt-Triton buffer as described just above. Three additional samples, each containing the same amount of actin as the first, were treated as follows: Actin (107 μM), initially in monomeric form, was incubated in the polymerization buffer described (15), with gelsolin (6.4 μM, one sample) or buffer (two samples), for 30 minutes at room temperature, to produce one sample with short, capped actin filaments (17) and two samples with full-length filaments (15). The sample with short, capped filaments, and one of the samples with full-length filaments, were stabilized by incubation with phallacidin (107 μM) (18) for an additional 60 minutes at room temperature. The remaining sample was incubated with buffer. After incubation, the three samples were diluted into low salt-Triton buffer for application to peptide affinity columns, as described above.

Affinity Chromatography. Synthetic CD peptides of the sequences indicated in Fig. 4a were purchased either from the Harvard Biopolymer Laboratory or from Research Genetics. Equimolar amounts of the peptides (2.2 micromoles) were coupled through their N-terminal cysteines to Thiopropyl Sepharose 6B resin (Pharmacia LKB). Coupling was carried out overnight at 4°C in high salt buffer (300 mM NaCl, 50 mM Tris acetate,10 mM EGTA, 2mM $MgCl_2$, pH 7.6). The reaction was

quantified by the appearance of 2-mercaptopyridine in the effluent of the resin column. This reporter group, released by the resin during coupling of sulfhydryl ligands, was measured by its absorbance at 343 nm (19). Usually, coupling of the peptides was about 70% efficient, yielding approximately 1.5 micromoles of coupled peptide per column. Variations up to two-fold were seen in a few experiments. However, in all experiments, coupled peptide was present in large molar excess (usually ~ 150-fold) compared to actin, which was applied as 10 nanomoles per column. After coupling, the columns were washed with low salt buffer. Either FAC proteins or purified actin were applied to the peptide-coupled columns overnight at 4°C in low salt-Triton extraction buffer. The columns were then washed with 50 vol of low salt buffer, followed by 5.3 vol of high salt buffer, to remove non-specifically bound proteins. Specifically bound proteins were then eluted by overnight incubation with 5.3 vol of 10mM dithiothreitol (DTT) in salt-free buffer (10mM Tris acetate, 2 mM EGTA, pH 7.6). DTT eluates were concentrated to about 0.65 ml using Centriprep 10 (Amicon) and adjusted to equal volumes with salt-free buffer. Because DTT breaks the disulfide bonds by which the peptides were coupled to the resin, elution with DTT favored the removal of peptide and all peptide-bound proteins from the columns.

SDS-PAGE. The DTT eluates were analyzed using either standard 10% or 15% reducing gels (13), or modified reducing gels containing 20% acrylamide and 10% glycerol (20). The modified gels resolved the small CD peptides as well as actin. Eluates of the radiolabeled FAC proteins were compared on the basis of equal amounts of radioactivity. Comparisons among eluates of purified unlabeled actin were made on the basis of aliquots representing equal fractions of the eluates.

Immunoprecipitation. This was done by standard methods (13), using a mouse monoclonal antibody reactive with all six known isoforms of vertebrate actin (Boehringer Mannheim), rabbit anti-mouse IgG (Sigma), and Pansorbin (Calbiochem).

Immunoblotting. This was performed as described (21). Rabbit polyclonal antiserum directed against the carboxy-terminal 21 amino acids of the α2 CD (22) was generously donated by Dr. Martin Hemler, Dana-Farber Cancer Institute, Boston, MA. Horseradish peroxidase conjugated goat anti-rabbit IgG (Calbiochem) served as the secondary antibody. Protein bands were visualized with diaminobenzidine and nickelous chloride (21).

RESULTS AND DISCUSSION

We isolated FAC proteins from metabolically labeled fibroblasts that bound to collagen-coated magnetic microbeads, using a recently described procedure (14). The radiolabeled FAC proteins were passed over α1 CD and α2 CD peptide affinity columns, and bound proteins were eluted and analyzed by SDS-PAGE and autoradiography. The total binding of labeled FAC proteins to α2 CD peptide was 10- to 50-fold greater than the binding to α1 CD peptide or to resin with no coupled peptide. Most of the FAC proteins larger than 18 kD bound preferentially to α2 CD peptide. By far the most prominent species showing such specific binding migrated with an apparent molecular weight of 42 kD (Fig. 1a and data not shown). This protein was subsequently identified as actin by immunoprecipitation (Fig. 1b). Many proteins in the FAC bind directly or indirectly to actin (4-8). Thus, our experimental result in Fig. 1a could represent direct binding of actin to the α2 CD peptide with indirect binding of other, actin binding FAC proteins, or the converse. We tested the hypothesis of direct actin binding by applying purified actin, free of other proteins, to α2 and α1 CD peptide columns as well as columns with no coupled peptide. Fig. 2a

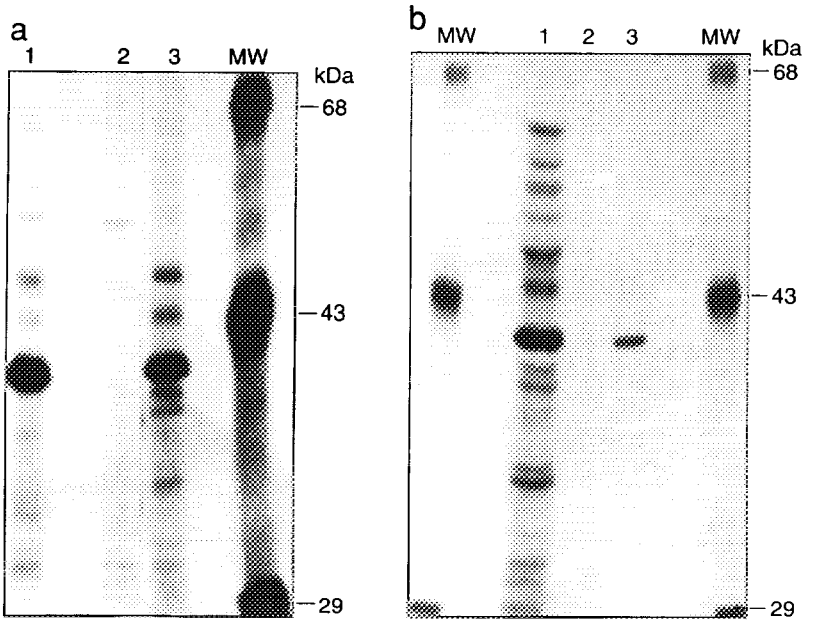


FIG. 1.

(a) Preferential binding of Focal Adhesion Complex (FAC) proteins to α2, as compared to α1, Cytoplasmic Domain (CD) peptide affinity columns, analyzed by SDS-PAGE and autoradiography. *Lane 1* : metabolically labeled FAC proteins (starting material); *Lane 2* : proteins eluted with DTT from the α1 CD peptide column; *Lane 3* : proteins eluted from the α2 CD peptide column.
(b) The 42kDa FAC protein eluted from the α2 CD peptide column is actin. FAC proteins eluted from an α2 CD peptide column were immunoprecipitated and analyzed by SDS-PAGE and autoradiography. *Lane 1*: eluate alone (without precipitation); *Lane 2* : immunoprecipitation with control antibody; *Lane 3* : immunoprecipitation with anti-actin antibody.

shows that actin bound specifically to the α2 CD peptide, while there was no detectable binding to the α1 CD peptide or to peptide-free resin.

Since the ionic conditions in these affinity column experiments favor polymerization of actin (16), we hypothesized that F actin was responsible for the observed binding. We therefore compared the binding of actin under the conditions used in Fig. 2a with that of explicitly polymerized actin (15), with and without subsequent treatment with phallacidin (which stabilizes actin in the F form) (18). In addition, one sample of actin was first polymerized in the presence of gelsolin to generate short filaments of F actin capped at the barbed ends (17), then stabilized with phallacidin. Fig. 2b indicates that F actin, stabilized as either full length or capped, short filaments, shows substantial binding to the α2 CD peptide.

In numerous experiments using different eluants, substantial elution of actin from the α2 CD peptide affinity resin was achieved only with dithiothreitol (DTT), a reagent that breaks disulfide bonds. Since peptides were coupled to the resin columns by such bonds, we expected to see both peptide and actin in the eluates (Fig. 2c). However, if DTT eluted actin and the α2 CD peptide as a tightly bound complex,

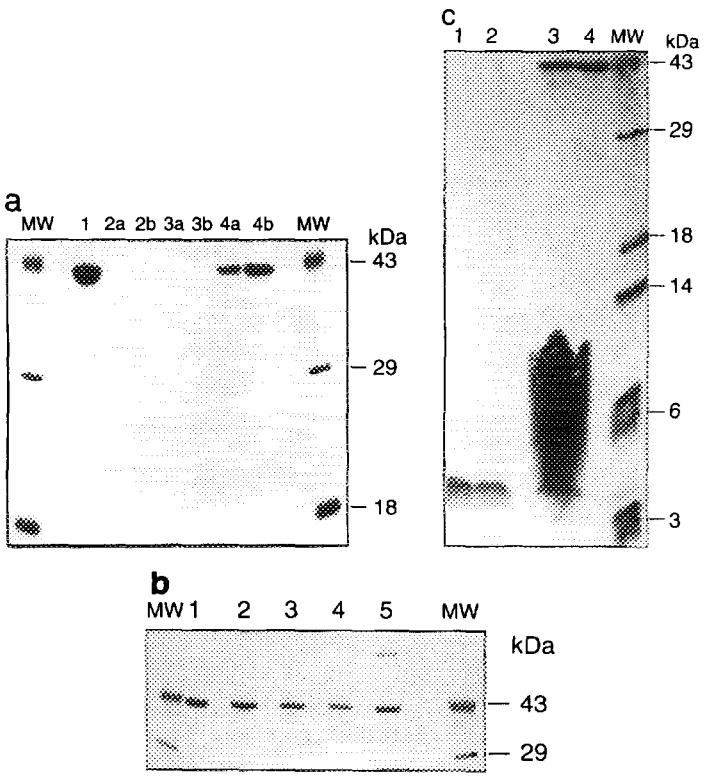


FIG. 2. *Specific binding of purified actin to α2 CD peptide.*

(a) Purified actin was applied to various peptide affinity columns and eluted with DTT. The eluates were subjected to SDS-PAGE and the gel was stained with Coomassie Blue and photographed. *Lane 1*: purified actin standard; *Lanes 2a and 2b* : 25 and 50 μl, respectively, of eluate from a column with no coupled peptide; *Lanes 3a and 3b* : 25 and 50 μl of eluate from an α1 CD peptide column; *Lanes 4a and 4b* : 25 and 50 μl of eluate from an α2 CD peptide column.

(b) Four samples of purified actin, incubated under varying conditions of polymerization and regulation of actin filament length, were applied to α2 CD peptide columns, and eluted with DTT. The eluates were electrophoresed, and the SDS-PAGE gel was Coomassie-stained and photographed. *Lane 1* : actin standard; *Lane 2* : eluate of a column receiving F actin assumed polymerized by the ionic conditions of the affinity chromatography. (The procedure for this actin sample was the same as for the samples represented in Figs. 2a, 2c, 3, and 4.); *Lane 3* : eluate of a column receiving explicitly polymerized F actin with no additional treatment; *Lane 4* : eluate of a column receiving explicitly polymerized F actin stabilized as full-length filaments with phallacidin; *Lane 5* : eluate of a column receiving explicitly polymerized F actin stabilized as short, capped filaments with gelsolin followed by phallacidin.

(c) Purified actin was applied to an α2 CD peptide affinity column, eluted with DTT, and analyzed by SDS-PAGE on a modified reducing gel capable of resolving small peptides as well as actin. The gel was stained with Coomassie Blue and photographed. *Lane 1* : 15 μg of α2 CD peptide standard; *Lane 2* : 1.5 μl of DTT eluate; *Lane 3* : 50 μl of the same eluate; *Lane 4* : purified actin standard (8 μg.). At the lower dose of eluate (*Lane 2*), the peptide appeared as a well-resolved band at the bottom of the gel, and insufficient actin was present to be visualized. The larger amount of eluate (*Lane 3*) was sufficient to reveal the presence of actin; however, the excessive α2 CD peptide present appeared as a large, poorly defined smear. A control experiment (data not shown) demonstrated that 500 μg of α2 CD peptide produced a gel pattern very similar to the lower band of *Lane 3*. Thus, both actin and a large amount of α2 CD peptide were detected in the eluate, commensurate with the large molar excess of peptide coupled to the affinity column.

both molecules should co-immunoprecipitate with antibodies monospecific for actin. Fig. 3 shows an experiment wherein DTT eluates (like those in Fig. 2c) were treated to remove unbound α2 CD peptide and were then immunoprecipitated with anti-actin antibodies. The immunoprecipitated pellets were subjected to SDS-PAGE, blotted to Immobilon P, and probed with an antiserum directed against α2 CD peptide. The material specifically precipitated from the eluate by anti-actin was strongly and specifically positive for the α2 CD peptide (*Lane 1*). This result indicates that the peptide and F actin coprecipitated from the eluate, strongly supporting the concept that the eluate contains a complex of peptide bound to actin. Striking additional evidence for such a complex is provided by the finding that even under the conditions of an SDS reducing gel, material migrating with an apparent molecular weight equal to the combined weights of actin (42 kD) and peptide (3.3 kD) was immunoreactive with antibodies to the α2 CD peptide (Fig. 3, top of *Lane 1*). Additional immunoreactive material migrated at the position of the peptide, suggesting that some of the actin-peptide complex dissociated under the conditions of the SDS-PAGE gel. In addition to coprecipitation, we also observed cosedimentation of α2 CD peptide and actin from DTT eluates after appropriate ultracentrifugation (23) (not shown). Taken together, these findings strongly suggested a high affinity interaction between α2 CD peptide and F actin.

To assess the specificity of this interaction, we examined whether removal of critical amino acids from the peptide would eliminate actin binding. We applied purified actin to affinity columns prepared with truncated peptides (Fig. 4a). Fig. 4b

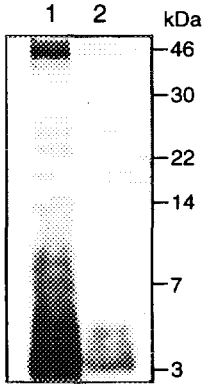


FIG. 3. Coprecipitation of α2 CD peptide and actin from eluates.

DTT eluates of actin from α2 CD peptide columns (as shown in Fig. 2c) were repetitively diluted with PBS and concentrated by Centricon 30 to remove unbound α2 CD peptide. These concentrated eluates were immunoprecipitated with anti-actin (*Lane 1*) or a control mAb (*Lane 2*), followed by protein G Sepharose. The immunoprecipitated pellets were subjected to SDS-PAGE as in Fig. 2c. The gel was blotted to Immobilon-P. The blot was probed with an antiserum directed against α2 CD peptide. A duplicate blot probed with normal rabbit serum as a control displayed no bands at the position of either the actin-peptide complex or the α2 CD peptide per se (data not shown).

a Amino Acid Sequences of Synthetic Peptides Coupled to Affinity Resins [1]

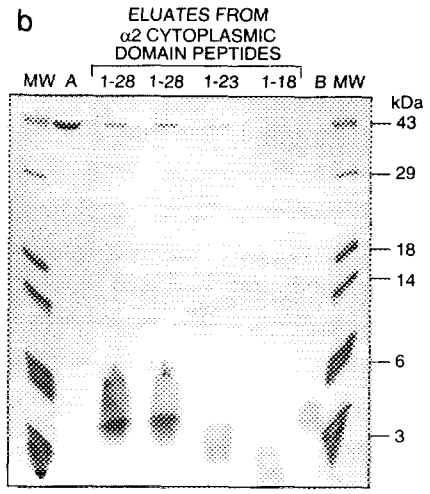
(A) Wild type sequences

Description	Amino Acid Sequence
α1 cytoplasmic domain (full-length, AA 1-16)	CKIGFFKRPLKKKMEK
α2 cytoplasmic domain (full-length, AA 1-28)	CKLGFFKRKYEKMTKNPDEIDETTELSS

(B) Truncated sequences

Description	Amino Acid Sequence
α2 cytoplasmic domain (truncated, AA 1-23)	CKLGFFKRKYEKMTKNPDEIDET
α2 cytoplasmic domain (truncated, AA 1-18, 24-28)	CKLGFFKRKYEKMTKNPDTELSS
α2 cytoplasmic domain (truncated, AA 1-18)	CKLGFFKRKYEKMTKNPD

[1] Note that all sequences are shown with an amino-terminal C, not part of the natural sequences, which was included to permit disulfide coupling to the affinity resin.



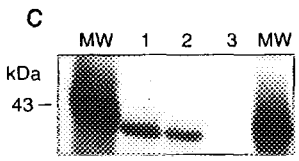


FIG. 4. Actin binding by full-length and truncated α2 CD peptides.

(a) Amino acid sequences of the α2 CD peptides tested. (All peptides contained an added N-terminal cysteine to permit disulfide coupling to the resin columns.)
(b) Affinity columns were prepared from two independent preparations of full-length α2 CD peptide and from the truncated peptides shown. Purified actin was applied and eluted, and the eluates electrophoresed, as in Fig. 2c. The gel was stained with Coomassie Blue and photographed. *Lane A* : actin standard; *Lane B* : full-length α2 CD peptide standard. Eluates from two columns of full-length α2 CD peptide (1-28), and one eluate each from columns with peptides truncated by five (1-23) and ten amino acids (1-18) are shown on this gel. The extent of actin binding to the indicated peptides is shown by the well-defined bands at the top of the gel. The poorly-defined patterns at the bottom of the gel represent large amounts of eluted peptides (see Fig. 2c).
(c) Affinity columns were prepared from two 23 amino acid truncated α2 CD peptides, one with a terminal deletion and one with an internal deletion, as shown in Fig. 4a, lines 3 and 4, respectively. Purified actin was applied and eluted with DTT, and the eluates electrophoresed. The gel was silver stained and photographed. *Lane 1*: actin standard; *Lane 2* : eluate from the 1-23 α2 CD peptide containing the sequence EIDET; *Lane 3* : eluate from the 19-23-deleted α2 CD peptide lacking the sequence EIDET.

shows that removal of 5 amino acids from the carboxy terminal of α2 CD peptide had only a minor effect on actin binding. However, removal of 10 amino acids reduced actin binding to undetectable levels. These results suggest that the 5 amino acid sequence EIDET (amino acids 19-23 of the α2 CD peptide) is critical for actin binding. To test the alternative possibility that reduced actin binding was a non-specific

consequence of decreased peptide length, we obtained a 23 amino acid peptide specifically lacking the sequence EIDET (Fig. 4a, line 4). An affinity column prepared from this hypothetically "negative 23-mer" in fact showed greatly reduced actin binding compared to a column prepared with the "positive 23-mer" including the sequence EIDET (Fig. 4a, line 3), as demonstrated in Fig. 4c. These results support the concept that the binding of actin to α2 CD peptide is specific. In an attempt to further analyze the requirements for the actin α2 CD peptide interaction, we did numerous experiments aimed at blocking the binding of actin to resin-bound α2 CD peptide using an excess of unbound peptide in solution. Unbound peptides included the full-length 28-mer, a 27-mer lacking the amino-terminal C, and the 5-mer EIDET. No blocking of actin binding was detected in any of these experiments. Similarly, the cosedimentation of actin and α2 CD peptide seen after interaction of actin with the bound peptide was not observed with the the free peptide.

We have shown that F actin can bind specifically to a peptide with the same amino acid sequence as the CD of α2 integrin, provided that the peptide is tethered at its N terminus. (In vivo, the N terminus is bound to the plasma membrane.) In our view, the simplest hypothesis consistent with all of our results is that the binding of actin to α2 CD peptide is strongly dependent on the appropriate conformation of the CD peptide. There is precedent for the conformation dependence of interactions between actin and actin binding proteins. A recent report notes that an F actin binding site on vinculin is masked until intramolecular associations between the head and tail domains of vinculin are disrupted (24). Since the amino acids PD immediately precede the critical sequence EIDET in α2 CD peptide (Fig. 4a, line 2), deletion of these amino acids may effectively disrupt a β turn, defined as several polar residues following a helix-breaking amino acid (P or Q) (25). Deletion of a 5 residue β turn from the CD of the αV integrin chain of αVβ3 was recently shown to result in alterations in ligand binding and conformational changes in the αVβ3 heterodimer (25). It is thus possible that the sequence EIDET is critical for actin binding because it imparts a specific conformation to the α2 CD peptide. Our consistent observations of striking differences between the resin-bound and the free peptide provide indirect support for the importance of conformation of the α2 CD peptide in actin binding.

The collagen binding integrin α2β1 has been implicated in several processes involving remodeling of fibrillar collagen matrices, including wound healing (10), vitreoretinal traction (1), scleroderma (26), cirrhosis (27), and pulmonary fibrosis (28). Our results suggest that the specific ability of α2β1 integrin (as contrasted to α1β1) to exert forces on fibrillar collagen matrices may derive from the capacity of the α2 CD to interact directly with F actin.

REFERENCES

1. Kupper, T. S. and Ferguson, T. A. (1993) *FASEB Journal* **7,** 1401-1406
2. Hynes, R. O. (1994) *Curr. Opin. Genet. Dev.* **4,** 569-574

3. Wang, N., Butler, J. P. and Ingber, D. E. (1993) *Science* **260,** 1124-1127
4. Miyamoto, S., Akiyama, S. K. and Yamada, K. M. (1995) *Science* **267,** 883-885
5. Otey, C. A., Vasquez, G. B., Burridge, K. and Erickson, B. W. (1993) *J. Biol. Chem.* **268,** 21193-21197
6. Hemler, M.E., Weitzman, J. B., Pasqualini, R., Kawaguchi, S., Kassner, P. D. and Berdichevsky, F. B. Structure, biochemical properties and biological functions of integrin cytoplasmic domains. In: Integrin: The Biological Problem. (editor, Takada, Y. , CRC Press, Ann Arbor, MI), in press
7. La Flamme, S. E., Thomas, L. A., Yamada, S. S. and Yamada, K. M. (1994) *J. Cell Biol.* **126,** 1287-1298
8. Clark, E. A. and Brugge, J. S. (1995) *Science* **268,** 233-239
9. Kawaguchi, S., Bergelson, J. M., Finberg, R. W. and Hemler, M. E. (1994) *Molec. Biol. Cell* **5,** 977-988
10. Schiro, J. A., Chan, B. M. C., Roswit, W. T., Kassner, P. D., Pentland, A. P., Hemler, M. E., Eisen, A. Z. and Kupper, T. S. (1991) *Cell* **67,** 403-410
11. Klein, C. E., Dressel, D., Steinmayer, T., Mauch, C., Eckes, B., Krieg, T., Bankert, R. B. and Weber, L. (1991) *J. Cell Biol.* **115,** 1427-1436
12. Chan, B. M. C., Kassner, P. D., Schiro, J. A., Byers, H. R., Kupper, T. S. and Hemler, M. E. (1992) *Cell* **68,** 1051-1060
13. Harlow, E. and Lane, D. Antibodies: A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1988)
14. Plopper, G. and Ingber, D.E. (1993) *Biochem. Biophys. Res. Commun.* **193,** 571-578
15. Hartwig, J. H., Thelen, M., Rosen, A., Janmey, P. A., Nairn, A. C. and Aderem, A. (1992) *Nature* **356,** 618-622
16. Hitchcock, S. E., Carlsson, L. and Lindberg, U. (1976) *Cell* **7,** 531-542
17. Weeds, A. and Maciver, S. (1993) *Curr. Opin. Cell Biol.* **5,** 63-69
18. Cooper, J. A. (1987) *J. Cell Biol.* **105,** 1473-1478
19. Stuchbury, T., Shipton, M., Norris, R., Malthouse, J. P. G., Brocklehurst, K., Herbert, J. A. L. and Suschitzky, H. (1975) *Biochem J.* **151,** 417-432
20. Giulian, G. G., Shanahan, M. F., Graham, J. M. and Moss, R. L. (1985) *Fed. Proc.* **44,** 686
21 Gallagher, S., Winston, S. E., Fuller, S. A., and Hurrell, J. G. R. In: Current Protocols in Molecular Biology Volume 2 (editors, Ausubel, F.M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. and Struhl, K.) 10.8.1-10.8.17 (Wiley, New York, 1994)
22. Takada, Y. and Hemler, M. E. (1989) *J. Cell Biol.* **109,** 397-407
23. Lo, S. H., Janmey, P. A., Hartwig, J. H. and Chen, L. B. (1994) *J. Cell Biol.* **125,** 1067-1075
24. Johnson, R. P. and Craig, S. W. (1995) *Nature* **373,** 261-264
25. Filardo, E. J. and Cheresh, D. A. (1994) *J. Biol. Chem.* **269,** 4641-4647
26. Kupper, T. S. (1995) *Intern. Rev. Immunol.* **12,** 283-291
27. Rudolph R., McClure, W. and Woodward, M. (1979) *Gastroenterol.* **76,** 704-709
28. Crouch, E. (1990) *Am. J. Physiol.* **259,** L159-184