Elements within the First 17 Amino Acids of Human Osteonectin Are Responsible for Binding to Type V Collagen*

(Received for publication, October 23, 1995, and in revised form, December 19, 1995)

Rong-Lin Xie and George L. Longt
From the Department of Biochemistry, University of Vermont, Burlington, Vermont 05405

The region in human osteonectin (ON) responsible for binding to type V collagen has been identified as the first 17 NH₂-terminal residues. This conclusion is based upon binding studies with deletion mutants of ON produced in Escherichia coli, in which parts of the first 17 amino acids have been removed. Wild-type ON from E. coli and mammalian cell-derived nonglycosylated ON bind identically to type V collagen and at least twice as effectively as mammalian cell-derived N-glycosylated ON. In previous studies, it was shown that N-glycosylation at residue 99 significantly reduces the capacity of ON to bind to type V collagen. Results reported in this communication demonstrate that the actual binding site on ON for type V collagen is distal from the site of N-glycosylation in terms of amino acid sequence but may be proximal in the folded, fully glycosylated, three-dimensional structure. Consistent with this conclusion is the ability of a synthetic peptide consisting of amino acids 1-17 to specifically inhibit the binding of ON to type V collagen.

Osteonectin (ON)\(^1\)/SPARC/BM-40 (1–3), a secreted, single-chain, acidic, Ca\(^{2+}\)-binding glycoprotein, is a major noncollagenous extracellular matrix protein in bone and dentine (1, 4) as well as in many normal and neoplastic human soft tissues (5) and cultured cells (6, 7). It is also synthesized, stored, and secreted by human blood platelets (8, 9). Earlier studies have suggested several functions for ON in the extracellular matrix, including the regulation of bone mineralization (1, 10), the control of cell shape (11), tissue remodeling or repair (12), cell migration (6), proliferation, and differentiation (12–16). Osteonectin from different sources binds differentially to different type collagens. Osteonectin from bovine bone binds to type I, III, and V collagen (1, 15, 17), whereas that from mouse parietal yolk sac cells binds only to type III and V collagen (18), and that from human platelets has no affinity for any of the three collagen types (17). The difference between bovine bone and human platelet ON binding to collagen has been attributed to differences in N-glycosylation (17). Osteoblast- and megakaryocyte-derived mRNA encoding ON are identical in size and restriction enzyme frustration patterns (19), lending further support to the hypothesis that differences in structure and collagen binding between bone and platelet-derived ON reside at the level of N-glycosylation. Osteonectin (BM-40) from mouse Engelbreth-Holm-Swarm tumor binds to type IV collagen but shows markedly reduced binding to type I, III, V, and VI collagen (20). The region of BM-40 binding to type IV collagen has been identified as the EF-hand and \(\alpha\)-helical domains in the carboxy-terminal half of BM-40 (21).

Mature human ON consists of 286 amino acids and contains two potential Asn-X-(Thr/Ser) N-glycosylation sites, located at positions 71 and 99 (22, 23). Recently, we reported that the binding site in human ON for type V collagen resides in the amino-terminal half (amino acids 1-146) of ON and that the capacity for type V collagen binding is significantly reduced by N-glycosylation at residue 99 (24). Residue 71 appears to have no or very little attached carbohydrate (24). In the present study, employing a set of deletion mutants, the binding region in ON has been further localized to the first 17 amino acids of the mature protein and appears to involve a tertiary structure comprising of several amino acids.

EXPERIMENTAL PROCEDURES

Materials—Type V human placental collagen, bovine serum albumin, isopropyl-1-thio-\(\beta\)-galactopyranoside, \(\alpha\)-dithiothreitol, phenylmethylsulfonyl fluoride, protamine sulfate, O-phenylendiamine, DEAE-Sephadex A-25, and goat anti-mouse antibody/peroxidase conjugate were all obtained from Sigma. Benzamidine hydrochloride hydrate was purchased from Aldrich. Luria broth base, expression vector pUC19, T4 ligase, and E. coli DH1 cells were made competent by CaCl₂ treatment, from Life Technologies, Inc. Horse anti-mouse IgG peroxidase conjugate was purchased from Vector Laboratories, Inc. (Burlingame, CA). Restriction endonuclease HindIII, EcoRI, and PvuII were obtained from New England Biolabs, Inc. (Beverly, MA). QIA Prep alkaline lysis kits were purchased from Qiagen Inc. (Chatsworth, CA). Anti-ON mouse monoclonal antibody II1A\(_{AB}\) and bovine bone ON were generous gifts from Dr. K. G. Mann (Burlington, VT).

Construction of Wild-type and Truncated Human Osteonectin Expression Vector and Site-specific Deletion Mutagenesis—Polymerase chain reaction (PCR)-enabled mutagenesis (25) was used to construct a pUC19-derived plasmid vector for the expression of wild-type amino-terminal half (amino acids 1-146) of human ON (thON) in E. coli. Advantage was taken of the multiple cloning site (MCS) within the expressible \(\beta\)-galactosidase gene of pUC19 (26). Consequently, thON and derived deletion mutants contain at their NH₂-termini seven amino acids of \(\beta\)-galactosidase (NH₂-(Met)-Thr-Met-Ile-Thr-Pro-Ser-Leu) and eight "zero" amino acids (Thr-Gly-Arg-Apa-Thr-Thr-Ser-COOH) at their COOH termini. Template for PCR-enabled mutagenesis was wild-type thON (amino acids 1–146) mammalian cell expression vector thON/pP5S, described elsewhere (24). Fig. 1A shows schematically the construction of type V collagen expression vector, and Fig. 1B shows the resulting recombinant protein products for wild-type and the six deletion mutants. Mutagenic primer B shown in Fig. 1A (5’-cagaagaagggGCCCCTCGAGAAGGCTGCC-3’) is composed of a five-nucleotide "clamp" followed by the inverse complement of amino acid codons 146–141 of ON and includes a naturally occurring EcorI digestion site (underlined). Mutagenic primers A consisted of the following 5’-caagaagggGCCCTCTAGAAGGCTGCC-3’. For wild-type thON (composed of a four-nucleotide clamp, seven nucleotides of the pUC19 MCS including a HindII digestion site (underlined) and one nucleotide.
Binding of Osteonectin to Type V Collagen

**Fig. 1. Construction and expression of recombinant osteonectin in E. coli.** Part A, schematic diagram of pUC19-derived wild-type truncated osteonectin expression vector. The mammalian cell expression vector hON/pDS, described in Ref. 24, was used as PCR template. PCR mutagenic primers A and B are described under "Experimental Procedures." RI, EcoRI; H3, HindIII; MCS, multiple cloning site. Segment lengths are not shown to scale. The relevant coding strand nucleotide and amino acid sequences in the expression vector obtained by PCR amplification RI and H3 digest gel purify frag (bottom).

**Gel Ligation** using as a single, resolved peak by HPLC, was checked for purity and represented with a zig-zag letter code. Name designations are described in the text. **Expression and Purification of Wild-type tHON and Deletion Mutants** from E. coli—Bacteria containing the correct mutant plasmid were grown in liquid LB media with 50 μg of ampicillin/ml at 37 °C in a shaker bath until the A600nm = 0.5. At this point isopropyl-1-thio-β-D-galactopyranoside (1 mM final concentration) was added to induce protein expression, and culture was continued for 6 h to achieve maximum expression. Cells were harvested by low speed centrifugation and lysed by sonication on ice (4 × 30 s) in cold 20 mM Tris, 5 mM EDTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 10 mM benzamidine, pH 7.4. The sonicate was centrifuged at 30,000 × g for 30 min to remove cell debris. Protamine sulfate in liquid form was slowly added with stirring to a final concentration of 0.1% to remove nucleic acids. After the addition of protamine sulfate, the solution was cleared by centrifugation at 30,000 × g for 30 min (27). Following this step, the supernatant containing ON was purified as described previously (24). The purified protein was analyzed by Western blotting and protein silver staining. Protein concentration was determined by Micro BCA protein assay (Pierce Inc. Rockford, IL) following the supplier’s instructions and using bovine bone osteonectin as a standard.

**Binding Assays—** The binding of different forms of ON to type V collagen and monoclonal antibody IIIA3A8 were performed by the ELISA method as described previously (24). The peptide corresponding to amino-terminal amino acids 1–17 of ON was synthesized on an Advanced Chemtech model 90 automatic peptide synthesizer using conventional Merrifield chemistry with β-butyloxycarbonyl amino acid N-derivatives and carbodiimide coupling. Following synthesis, the peptide was cleaved from the solid support resin with HF in trifluoroacetic acid and purified by HPLC reverse-phase chromatography. The peptide, migrating as a single, resolved peak by HPLC, was checked for purity and composition by conventional amino acid analysis and mass analysis on a PerSeptive Biosystems Voyager time-of-flight mass spectrometer and shown to be at least 80–85% pure. A control peptide of the same size and similar charge properties, SNNGNRRNYY-D, was synthesized and characterized by the above methods.

**ELISA Assay of Wild-type tHON, Δ9-17 tHON, and Peptide Competitive Binding to Collagen V—** An ELISA method based on the supplier's peptide/DNA mapping as a single, resolved peak by HPLC, was checked for purity and composition by conventional amino acid analysis and mass analysis on a PerSeptive Biosystems Voyager time-of-flight mass spectrometer and shown to be at least 80–85% pure. A control peptide of the same size and similar charge properties, SNNGNRRNYY-D, was synthesized and characterized by the above methods.

**ELISA Assay of Wild-type tHON, Δ9-17 tHON, and Peptide Competitive Binding to Collagen V—** An ELISA method based on the supplier’s peptide/DNA mapping as a single, resolved peak by HPLC, was checked for purity and composition by conventional amino acid analysis and mass analysis on a PerSeptive Biosystems Voyager time-of-flight mass spectrometer and shown to be at least 80–85% pure. A control peptide of the same size and similar charge properties, SNNGNRRNYY-D, was synthesized and characterized by the above methods.
with its predicted 161 amino acid size. As shown in Fig. 1, band upon 12% SDS-PAGE under reducing conditions (Fig. 1).

Each of the purified proteins showed a single

mutations had been introduced by PCR amplification (data not shown).

Construction of Wild-type tHON and Deletion Mutant Expression Vectors—CDNA sequencing and endonuclease digestion of wild-type and mutant vectors confirmed that the desired constructs had been accomplished and that no inadvertent mutations had been introduced by PCR amplification (data not shown).

Expression, Purification, and Properties of Wild-type tHON and Mutants—Each of the purified proteins showed a single band upon 12% SDS-PAGE under reducing conditions (Fig. 1C). Wild-type chimeric tHON migrates in a manner consistent with its predicted 161 amino acid size. As shown in Fig. 1C, each of the deletion mutants moves slightly faster than wild-type ON. Amino acid sequencing of purified forms of ON confirmed that the NH₂ terminii, including the deletion regions, were as expected (data not shown).

Affinity of Antibody IIIA₃A₈ for Different ONs. Osteonectin-antibody complexes were detected immunologically as described in the text. tHON (●), Δ₉₋₁₅ (●), Δ₅₋₁₅ (●), Δ₁₂₋₁₇ (●), Δ₃₋₁₇ (●), and Δ₁₇ (●). Error bars represent the range in values from three independent determinations.

instructions (Hematologic Technologies Inc. Essex Junction, VT) was used to assay ONs and peptide competitive binding to collagen V. Type V collagen from human placenta was diluted to 10 μg/ml in 50 mM NaHCO₃ (pH 9.7) and applied in 0.1-ml volumes to ELISA plates (Corning, NY) for 3 h. The wells were then washed with 20 mM Tris (pH 7.4), 150 mM NaCl, 2 mM CaCl₂, 0.05% (v/v) Tween 20 (TBS/Tween/CaCl₂) buffer. The wells were then blocked with 100 μl of 2% bovine serum albumin, 20 mM Tris (pH 7.4), 150 mM NaCl for 1 h at room temperature. Following blocking, the wells were washed 2 times each with 200 μl of TBS/Tween buffer. Wild-type tHON, Δ₁₇ tHON, and peptides were diluted in TBS/Tween/CaCl₂ buffer, and 50 μl of 0.25 μg/μl ON was applied to each well, immediately after which 50 μl of different concentrations of peptide was applied. After incubation for 1 h at 4 °C, the wells were washed 3 times each with 200 μl of TBS/Tween/CaCl₂ buffer, followed by the addition of 100 μl of 1° monoclonal antibody IIIA₃A₈ and washing. Then 2° antibody, goat anti-mouse/ peroxidase conjugate diluted 1:3000, was added to each well and incubated for 1 h. After washing, enzyme substrates (100 μl 50 mM citrate buffer, and peptides were diluted in TBS/Tween/CaCl₂ buffer, and 50 μl of 0.25 μg/μl ON was applied to each well, immediately after which 50 μl of different concentrations of peptide was applied. After incubation for 1 h at 4 °C, the wells were washed 3 times each with 200 μl of TBS/Tween/CaCl₂ buffer, and the absorbance at 490 nm was measured with a Vmax spectrophotometer (Molecular Devices Co., Menlo Park, CA).

RESULTS

Construction of Wild-type tHON and Deletion Mutant Expression Vectors—CDNA sequencing and endonuclease digestion of wild-type and mutant vectors confirmed that the desired constructs had been accomplished and that no inadvertent mutations had been introduced by PCR amplification (data not shown).

Expression, Purification, and Properties of Wild-type tHON and Mutants—Each of the purified proteins showed a single band upon 12% SDS-PAGE under reducing conditions (Fig. 1C). Wild-type chimeric tHON migrates in a manner consistent with its predicted 161 amino acid size. As shown in Fig. 1C, each of the deletion mutants moves slightly faster than wild-type ON. Amino acid sequencing of purified forms of ON confirmed that the NH₂ terminii, including the deletion regions, were as expected (data not shown).

Affinity of Antibody IIIA₃A₈ for Different ONs. Osteonectin-antibody complexes were detected immunologically as described in the text. tHON (●), Δ₉₋₁₅ (●), Δ₅₋₁₅ (●), Δ₁₂₋₁₇ (●), Δ₃₋₁₇ (●), and Δ₁₇ (●). Error bars represent the range in values from three independent determinations.

site-directed mutagenesis (Asn⁹⁹ → Gln; N99Q) significantly increased the binding to type V collagen (24). Fig. 3 compares the binding of E. coli and mammalian cell-derived wild-type tHONs and the N99Q mutant to type V collagen. The curves indicate that nonglycosylated wild-type tHON from E. coli has the same affinity for type V collagen as the nonglycosylated N99Q mutant and that both have enhanced binding compared with glycosylated wild-type tHON. These results confirm that the difference in type V collagen binding between the mammalian cell-derived wild-type and N99Q tHON is due to the lack of carbohydrate and not due to the amino acid substitution itself, since the presence of Asn at position 99 (E. coli material) versus Gln (N99Q mutant) has no apparent effect. These results also demonstrate that ON produced in E. coli binds equivalently to that from mammalian cells. Furthermore, the results demonstrate that the seven-amino acid NH₂-terminal β-galactosidase and eight-amino acid COOH-terminal “zeno” extension peptides have no apparent effect on tHON binding to type V collagen.

Binding of the Deletion Mutants to Type V Collagen—Results shown in Fig. 4 indicate that the ability of wild-type tHON to bind type V collagen is significantly affected by deletions in the NH₂-terminal region. Deletion of the first four (Δ4) or eight (Δ8) NH₂-terminal residues of tHON reduces the binding capacity by 73 and 91%, respectively. Further deletion (Δ12 and Δ17) showed only slight binding above background. These results suggest that the first four residues of ON play an important role in type V collagen binding. However, as shown in Fig. 4, the internal deletion mutants of residues 5–8 (Δ₅₋₈) and 9–12 (Δ₉₋₁₂) also exhibit severely reduced (if any) binding. Taken together, the mutant results clearly indicate that the NH₂-terminal 17 amino acid region of ON is the major contributor to the binding interaction between ON and type V collagen; the binding site on ON is not just a unique amino acid residue or a few adjacent residues, but rather appears to involve an extended conformational interaction or distal binding residues within the first 17 amino acids.

Competitive Inhibition Assay of Amino Acids 1–17 Peptide Binding to Type V Collagen—As an independent confirmation of the ability of the amino acids 1–17 region of ON to bind to type V collagen, the effect on binding of a peptide representing
amino acids 1–17 was studied. The results, presented in Fig. 5, indicate that the peptide competitively blocks the binding of tHON to type V collagen, with a $K_I = 10 \mu M$ (50% inhibition). In contrast, the control peptide over the same concentration range (5–100 $\mu M$) had no effect. One possible explanation considered by us for the loss of binding by the deletion mutants was the necessary participation of an amino acid segment in the 1–17 region and a segment(s) in the distal 18–146 region. To test the possibility of complementation of binding, the effect of adding amino acids 1–17 peptide to $\Delta_{17}$ mutant protein was also measured. As Fig. 5 indicates, the addition of the peptide did not facilitate the binding of the $\Delta_{17}$ mutant ON. These results suggest the absence of co-participation by a second distal segment in type V collagen binding.

**DISCUSSION**

Earlier studies indicate that bovine bone and human bone ON bind to type V collagen (4, 17). Osteonectin frommouse parietal yolk sac cells also binds to type V collagen (18). In addition, studies with a truncated form of human ON containing only the amino-terminal half of the protein effectively binds to type V collagen, demonstrating that the binding region of ON resides within amino acid residues 1–146 (24). Based upon differences in glycosylation patterns as well as lectin binding properties, it was proposed that the collagen binding specificity of bone and platelet ON is related to differences in glycosylation (16). In order to further understand the region of ON involved in collagen binding and the effects of specific N-glycosylation sites on biological activity, we have demonstrated that after removal of oligosaccharide chain structures from bovine bone and human platelet ON by N-glycanase, their ability to bind to type V collagen is increased to an equal level. In addition, the results of site-specific mutagenesis at each of the two potential Asn-X-Thr glycosylation sites (amino acids 71 and 99) in ON indicate that only glycosylation at residue 99 affects type V collagen binding activity (24). Further studies using deletion mutants reported here demonstrate that the binding site in ON for type V collagen is in the first 17 amino acids of the protein. This segment is distal from the N-glycosylation site in regard to the amino acid sequence, but due to protein folding, and the spatial umbrella of the carbohydrate, may be conformationally proximal to the site of N-linked carbohydrate. No information relating to the three-dimensional structure of ON is available to further interpret our results. A second important conclusion reached from this study involving a family of related deletion mutants is that it does not appear that a unique amino acid residue or short segment of amino acids is capable by itself of directing effective ON binding to type V collagen, but rather, several small segments and/or an overall conformational state in the first 17 residues is necessary.

As indicated in Fig. 3 and reported in our earlier studies (24), tHON achieves half-maximum binding at $\sim 100 \mu M$, representing approximately 10-fold greater affinity for type V collagen than the blocking peptide ($K_I = 10 \mu M$). Differences of this order of magnitude are commonly observed for synthetic peptides versus the native protein and are thought to be largely due to the greater flexibility and conformational heterogeneity of the synthetic peptide. Despite the difference in affinity, the fact that complete inhibition can be accomplished by the peptide alone and the absence of binding in the presence of peptide plus $\Delta_{17}$ ON mutant protein strongly suggest that the binding of ON to type V collagen is recapitulated by the peptide.

Type V collagen is particularly abundant in vascular tissue, primarily due to its synthesis in smooth muscle cells. Smooth muscle cells and their protein products, including possibly type V collagen, are believed to play an important role in the development of atherosclerotic plaque. In human atherosclerotic lesions, the ratio of type V collagen to other types is elevated relative to the normal situation (28, 29). Additionally, two reports indicate that type V collagen can have a procoagulant effect (30, 31). This might result from the disruption or destruction of vascular endothelial cells at sites of tissue injury or remodeling, and consequential exposure of type V collagen by underlying smooth muscle cells. Kelm et al. (32) have reported that osteonectin binds to plasminogen and enhances tissue plasminogen activator conversion of plasminogen to plasmin. Both plasmin and tissue plasminogen activator are important thrombolytic agents. Kelm and co-workers (32) also reported the mediation of plasminogen binding to type V collagen by bovine bone osteonectin (32). Consequently, osteonectin, by serving as a bridge between type V collagen and thrombolytic agents, may play an important role in hemostasis in the absence of functional endothelium.

**Acknowledgments—**We are grateful to the laboratory of K.G. Mann (University of Vermont) for reagent materials listed in “Experimental Procedure,” and to Dr. William Church (University of Vermont) for the...
synthesis and purification of peptides, and for advice on the competitive peptide binding studies.

REFERENCES

1. Termine, J. D., Kleinman, H. K., Whitson, S. W., Conn, K. M., McGarvey, M. L., and Martin, G. R. (1981) Cell 26, 99–105
2. Sage, H., Johnson, C., and Bornstein, P. (1984) J. Biol. Chem. 259, 3993–4007
3. Dziadek, M., Paulsson, M., Aumailley, M., and Timple, R. (1986) Eur. J. Biochem. 161, 453–464
4. Romberg, R. W., Werness, P. G., Lollar, P., Riggs, B. L., and Mann, K. G. (1985) J. Biol. Chem. 260, 2728–2736
5. Porter, P. L., Sage, E. H., Lane, T. F., Funk, S. E., and Gown, A. M. (1995) J. Histochem. Cytochem. 43, 791–800
6. Sage, E. H., and Bornstein, P. (1991) J. Biol. Chem. 266, 14831–14834
7. Tremble, P. M., Lane, T. F., Sage, E. H., and Werb, Z. (1993) J. Cell Biol. 121, 1433–1444
8. Stenner, D. D., Tracy, R. P., Riggs, B. L., and Mann, K. G. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 6892–6896
9. Kelm, R. J., Jr., and Mann, K. G. (1990) Blood 75, 1105–1113
10. Otsuka, K., Yao, K. L., Wasi, S., Tung, P., Aubin, J. E., Sodek, J., and Termine, J. D. (1984) J. Biol. Chem. 259, 9805–9812
11. Everett, E. A., and Sage, E. H. (1992) Exp. Cell Res. 199, 134–146
12. Sage, E. H., Vernon, R. B., Decker, J., Funk, S., and Iruela-Arispe, M. L. (1989) J. Histochem. Cytochem. 37, 819–829
13. Mason, I. J., Murphy, D., Münke, M., Francke, U., Elliot, R., and Hogan, B. L. M. (1986) EMBO J. 5, 1831–1837
14. Holland, P. W. H., Harper, S. J., McVey, J. H., and Hogan, B. L. M. (1987) J. Cell Biol. 105, 473–482
15. Wewer, U. M., Albrechtsen, R., Fisher, L. W., Young, M. F., and Termine, J. D. (1988) Am. J. Pathol. 132, 345–355
16. Nomura, S., Willis, A. J., Edwards, D. R., Heath, J. K., and Hogan, B. L. M. (1988) J. Cell Biol. 106, 441–450
17. Kelm, R. J., Jr., and Mann, K. G. (1991) J. Biol. Chem. 266, 9632–9639
18. Sage, E. H., Vernon, R. B., Funk, S. E., Everett, E. A., and Angelia, J. (1989) J. Cell Biol. 109, 341–356
19. Villarreal, X. C., Grant, B. W., and Long, G. L. (1991) Blood 78, 1216–1222
20. Mayer, U., Aumailley, M., Mann, K., Timpl, R., and Engel, J. (1991) Eur. J. Biochem. 190, 141–150
21. Pottgiesser, J., Maurer, P., Mayer, U., Nischt, R., Mann, K., Timpl, R., Krieg, T., and Engel, J. (1994) J. Mol. Biol. 238, 563–574
22. Swaaroop, A., Hogan, B. L. M., and Francke, U. (1988) Genomics 2, 37–47
23. Villarreal, X. C., Mann, K. G., and Long, G. L. (1989) Biochemistry 28, 6483–6491
24. Xie, R. L., and Long, G. L. (1995) J. Biol. Chem. 270, 23212–23217
25. Nelson, R. M., and Long, G. L. (1989) Anal. Biochem. 180, 147–151
26. Yanisch-Perron, C., Vieira, J., and Messing, J. (1985) Gene(Amst.) 33, 103–119
27. Fränkly, C., Harris, D., and Moras, D. (1994) J. Mol. Biol. 241, 275–277
28. Oshima, A. (1981) Science 213, 666–668
29. Murata, K., Motayama, T., and Kotake, C. (1986) Atherosclerosis 60, 251–262
30. Kawamoto, Y., and Kaibara, M. (1990) Biochim. Biophys. Acta 1035, 361–368
31. Zilts, N. P., and Anderson, J. M. (1993) J. Vasc. Surg. 17, 710–718
32. Kelm, R. J., Jr., Swords, N. A., Orfeo, T., and Mann, K. G. (1994) J. Biol. Chem. 269, 30147–30153
33. Laemmli, U. K. (1970) Nature 227, 680–685
Elements within the First 17 Amino Acids of Human Osteonectin Are Responsible for Binding to Type V Collagen
Rong-Lin Xie and George L. Long

J. Biol. Chem. 1996, 271:8121-8125.
doi: 10.1074/jbc.271.14.8121

Access the most updated version of this article at http://www.jbc.org/content/271/14/8121

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 33 references, 15 of which can be accessed free at http://www.jbc.org/content/271/14/8121.full.html#ref-list-1