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Two Collagen-binding Domains of Vitronectin

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ABSTRACT. **Vitronectin is a cell-adhesive glycoprotein present in animal blood and extracellular matrix. To establish the molecular basis of vitronectin interactions with extracellular matrix macromolecules, the binding site of vitronectin to collagen has been investigated. Vitronectin fragments obtained by formic acid cleavage were separated by heparin-affinity chromatography followed by gel filtration chromatography. The collagen-binding activity of the fragments was assayed in terms of inhibitory activity on the binding of ^{125}I-vitronectin to immobilized collagen. There were two groups of collagen-binding fragments. One group consisted of 5 heparin-binding fragments with estimated molecular masses of 12 kDa, 14 kDa, 16 kDa, 18 kDa, and 19 kDa in SDS-polyacrylamide gel electrophoresis. The other group consisted of 2 heparin-nonbinding fragments migrating at 18 kDa and 40 kDa. These results indicate that there are two collagen-binding sites in the vitronectin molecule; one located close to the heparin-binding domain in the COOH-terminal half and the other located in the NH_2-terminal half of vitronectin.**

Vitronectin is a cell-adhesive glycoprotein present in animal blood and extracellular matrix (for reviews, see 1–3). Vitronectin in animal sera or in physiological salt solution spontaneously deposits on the surface of tissue culture dishes *in vitro*, attaches cells to the substratum, changes the shape of the cells (4–6), and promotes cell migration (7, 8). The principal active site is a tripeptide, Arg-Gly-Asp, located close to the NH_2-terminus of vitronectin (9, 10). Cell surface vitronectin receptor, mainly integrin $\alpha_V\beta_3$, interacts with vitronectin through this tripeptide (11, 12). Treatment of melanoma cells with anti-integrin $\alpha_V\beta_3$ antibodies results in stimulation of both secretion and transcription of type IV collagenase (13). Therefore, the vitronectin-integrin $\alpha_V\beta_3$ system seems to be a cell-surface regulatory system for attachment, shape, migration, secretion, and gene expression of cells. At a higher level, this system itself is regulated by soluble factors such as tumor necrosis factor α, interferon γ, and transforming growth factor-β1 (14, 15). In addition, vitronectin plays a role in the regulation of blood coagulation and complement-mediated cytolysis in the soluble phase (for reviews, see 1, 2).

Immunohistochemical studies have revealed vitronectin deposits in loose connective tissues of skeletal muscle, embryonal lung, fetal membrane, and kidney (16, 17). Vitronectin is also found in vascular wall (18) and around elastic fibers in normal skin (19) as well as skin from patients with elastic tissue disorders (20). In human cancer patients, vitronectin is enriched in the stroma of breast and colon carcinomas (21) and in the parenchyma of glioblastoma tumor (22).

There should be macromolecules responsible for the deposition of vitronectin on tissue extracellular matrix. Glycosaminoglycans are likely candidates, since heparin strongly binds to vitronectin (16, 23, 24). Tissue type glycosaminoglycans including chondroitin sulfate, dermatan sulfate, hyaluronan, heparan sulfate, and keratan sulfate do not, however, seem to bind vitronectin (23, 24). On the other hand, Gebb *et al.* (25) reported that purified vitronectin binds to native collagen *in vitro*. The binding is quite strong, although it is inhibited by high salt concentration, and nothing is yet known about the colocalization of vitronectin with collagen *in vivo*. Endogenous plasma/serum vitronectin does not bind to collagen. Specific activating conditions, such as boiling after the addition of 8 M urea and heparin (26), are necessary for the acquisition of collagen-binding activity. Therefore, the physiological meaning of the collagen-binding ability of vitronectin is uncertain. But collagen-binding seems to be one of the essential properties of vitronectin, since various purified animal blood vitronectins, except chick yolk vitronectin (27), bind to collagen (28).

Therefore, we decided to probe the *in vitro* interaction of vitronectin with collagen by isolating the collagen-binding fragments of vitronectin, in order to clarify the collagen-binding site of vitronectin and to provide tools to investigate further the molecular basis of the in-

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teraction between vitronectin and collagen.

MATERIALS AND METHODS

Vitronectin and vitronectin fragments. Vitronectin was purified from human outdated plasma according to Yatohgo *et al.* (29). Purified vitronectin was extensively dialyzed against 10 mM NH_4HCO_3 and then lyophilized. Vitronectin (17.2 mg) was cleaved with 17 ml of 70% formic acid at 50°C for 16 h according to Suzuki *et al.* (30). Formic acid was removed by lyophilization after addition of 9 vol of distilled water. The vitronectin fragments were taken up in 0.13 M NaCl, 8 M urea, 5 mM EDTA, 10 mM β-mercaptoethanol, and 10 mM Na-phosphate buffer (pH 7.7), and applied to a heparin-Sepharose 4B column (5 ml bed volume). The flow-through fractions were collected, then heparin-binding fragments were eluted from the column with 0.5 M NaCl, 8 M urea, 5 mM EDTA, 10 mM β-mercaptoethanol, and 10 mM Na-phosphate buffer (pH 7.7). Both heparin-binding and -nonbinding fragments were concentrated and fractionated by gel filtration on a column of Sephacryl S-200 (16 mm × 94 cm) in 0.05 M NaCl, 8 M urea, 5 mM EDTA, 10 mM β-mercaptoethanol, and 10 mM Na-phosphate buffer (pH 7.7) at a flow rate of 0.065 ml/min/cm² at room temperature.

Collagen-binding assay. Binding of vitronectin to collagen was measured by using ^{125}I-labeled vitronectin and microtiter plates bearing immobilized collagen as described previously (31). Collagen-binding ability of vitronectin fragments was assayed in terms of inhibitory activity on the binding of ^{125}I-labeled whole vitronectin to collagen. Briefly, native type I collagen from porcine skin (Nitta Gelatin, Osaka, Japan) was incubated at a concentration of 10 μg/ml in 96-well soft microtiter plates made of polyvinyl chloride at 4°C overnight. The plates were rinsed, and blocked with 0.2% skim milk. Fifty μl of ^{125}I-labeled vitronectin in 0.05% Tween-20 and 10 mM Na-phosphate buffer (pH 7.4) was then added to each well. To examine the collagen-binding activity of vitronectin fragments, the fragments (0–50 μg/ml) were mixed with 0.5 μg/ml of ^{125}I-labeled vitronectin at this step. To examine the activity of Sephacryl S-200 fractions, each fraction was diluted to 1/20 with 0.05% Tween-20 and 10 mM Na-phosphate buffer (pH 7.4) after dialysis against 8 M urea, 5 mM EDTA, and 10 mM Na-phosphate buffer (pH 7.4). After incubation at 37°C for 1 h, the plates were rinsed 4 times with 0.05% Tween-20 and 10 mM Na-phosphate buffer (pH 7.4), and cut into individual wells with scissors. The radioactivity that remained in each well was counted with a gamma counter, Aloka ARC-500.

RESULTS

Vitronectin fragmentation and collagen-binding. Vitronectin (a mixture of 75 kDa and 65 kDa; Fig. 1, lane 1) was cleaved by 70% formic acid into many fragments ranging in size from 12 kDa to 70 kDa (Fig. 1, lane 2). Formic acid fragments of vitronectin interfered with the binding of ^{125}I-labeled vitronectin to collagen, though they were severalfold less active than intact vitronectin (Fig. 2). Thus, these fragments retained their collagen-binding activity. Vitronectin fragments were separated by heparin-affinity chromatography into heparin-nonbinding fragments (Fig. 1, lane 3) and heparin-binding fragments (Fig. 1, lane 4). In SDS-polyacrylamide gel electrophoresis, major heparin-nonbinding fragments migrated to positions corresponding to 40 kDa, 39 kDa, 33 kDa, 18 kDa, and 15 kDa, while major heparin-binding fragments migrated to 32 kDa, 23–26 kDa, 20 kDa, 19 kDa, 18 kDa, 16 kDa, 14 kDa, and 12 kDa. Both heparin-nonbinding and -binding fragments were examined for their ability to interfere with ^{125}I-labeled vitronectin binding to collagen. The mixture of heparin-binding fragments had activity as strong as that of intact vitronectin. The mixture of heparin-nonbinding fragments also had similar, but weaker, activity (Fig. 2).

Collagen-binding of heparin-binding fragments. Heparin-binding fragments were fractionated by gel filtration chromatography on Sephacryl S-200 in the pre-

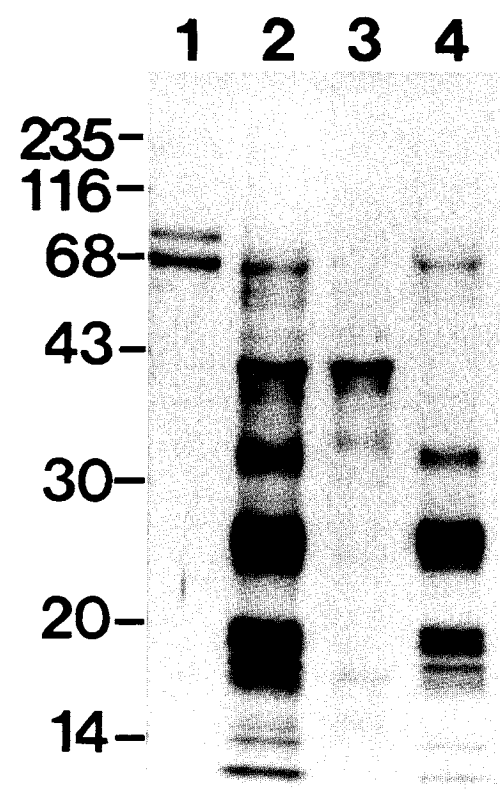

Fig. 1. SDS-polyacrylamide gel electrophoresis of vitronectin fragments after separation by heparin affinity chromatography. Human vitronectin (lane 1) was cleaved with 70% formic acid at 50°C for 16 h (lane 2) and then separated into heparin-nonbinding (lane 3) and heparin-binding fractions (lane 4) by heparin-affinity column chromatography. Molecular mass (in kDa) is indicated at the left.

sence of 8 M urea. Each fraction was tested for ability to interfere with the binding of ^{125}I-labeled vitronectin to collagen, as well as protein concentration. There was an interfering activity around fraction number 77, which corresponded to the third peak of the elution profile in terms of absorbance at 280 nm (Fig. 3). SDS-polyacrylamide gel electrophoresis of serial fractions from 70 to 82 revealed several bands (Fig. 4). The profile of the activity in Fig. 3 corresponds well to the appearance of 5 bands of less than 19 kDa in Fig. 4. Their molecular masses were 12 kDa, 14 kDa, 16 kDa, 18 kDa, and 19 kDa. These results suggest that one collagen-binding site of vitronectin is contained in these five heparin-binding fragments.

Collagen-binding of heparin-nonbinding fragments. Heparin-nonbinding fragments were similarly fractionated by gel filtration chromatography. Five major fractions were pooled and examined for collagen-binding activity and for polypeptide composition by SDS-polyacrylamide gel electrophoresis. Fractions 4 and 1 had relatively strong activity and fraction 2 had weak activity, while fractions 3 and 5 had no activity (Fig. 5A). Fraction 4 contained mainly an 18 kDa polypeptide and fraction 1 contained only a 40 kDa band, which was also present in fraction 2 (Fig. 5B). These results suggest that active fragments among the heparin-nonbinding fragments are those with molecular masses of 40 kDa

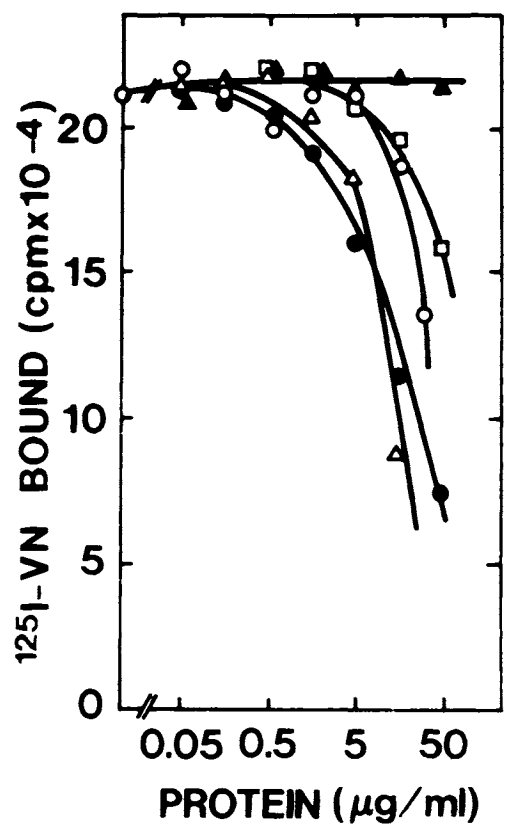

Fig. 2. Collagen-binding assay of vitronectin and vitronectin fragments. Collagen-binding activity was measured in terms of ability to inhibit the binding of ^{125}I-labeled vitronectin (^{125}I-VN) to microtiter plates bearing immobilized collagen. Increasing concentrations of vitronectin or vitronectin fragments were examined; vitronectin (●), formic acid digest of vitronectin (○), heparin-nonbinding fragments (□), heparin-binding fragments (△), and bovine serum albumin (▲) as a control.

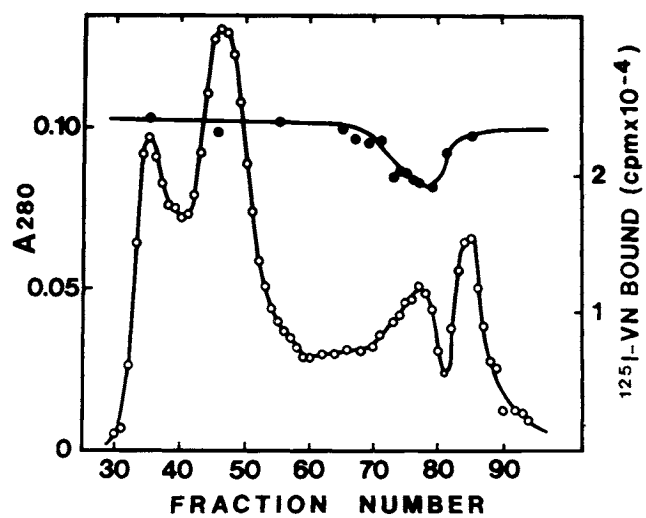

Fig. 3. Gel filtration of heparin-binding fragments of vitronectin. Heparin-binding fragments of vitronectin were chromatographed on a Sephacryl S-200 column (16 mm × 94 cm) in 0.05 M NaCl, 8 M urea, 5 mM EDTA, 10 mM β-mercaptoethanol, and 10 mM Na-phosphate buffer (pH 7.7). Each fraction was assayed for protein concentration in terms of absorption at 280 nm (○) and collagen-binding activity (●).

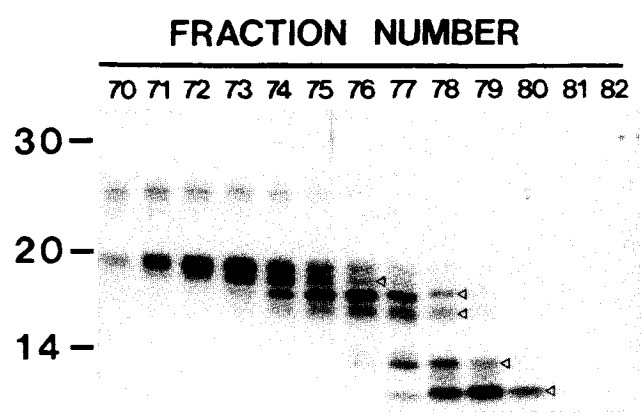

Fig. 4. SDS-polyacrylamide gel electrophoresis of heparin-binding fragments of vitronectin. Fractions 70 to 82 in Fig. 3, spanning the peak of collagen-binding activity, were examined for protein composition by SDS-polyacrylamide gel electrophoresis. Open triangles indicate collagen-binding fragments of 12 kDa, 14 kDa, 16 kDa, 18 kDa, and 19 kDa bands. Molecular mass (in kDa) is indicated at the left.

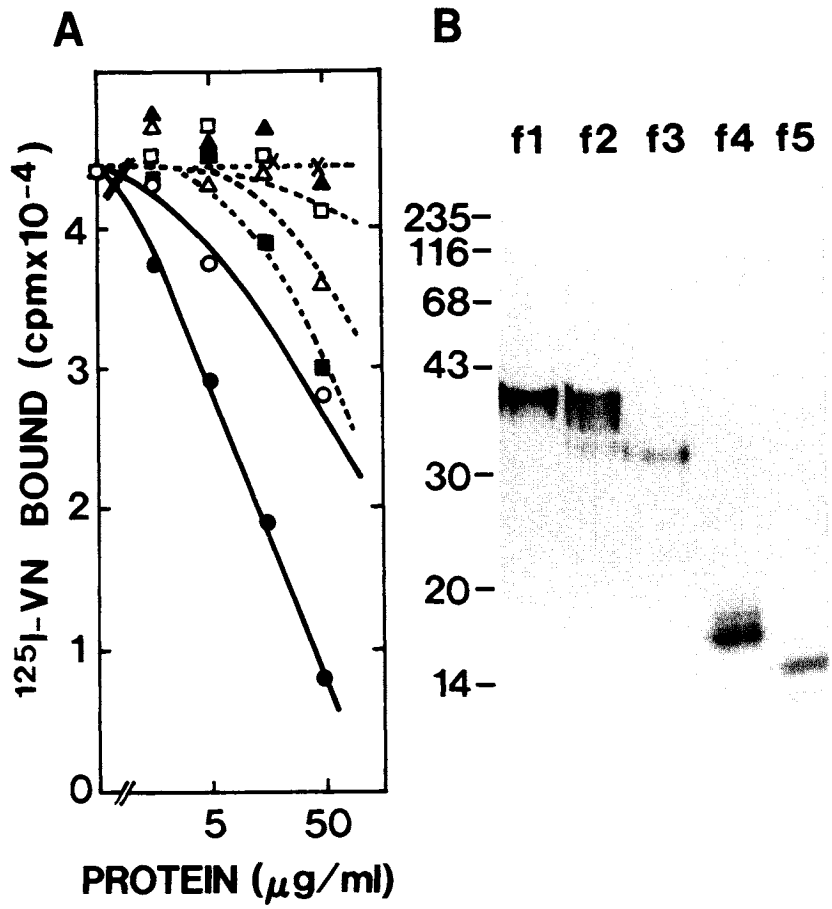

Fig. 5. Collagen-binding activity (A) and SDS-polyacrylamide gel electrophoresis (B) of heparin-nonbinding fragments of vitronectin. Heparin-nonbinding fragments of vitronectin were further separated by Sephacryl S-200 column chromatography. (A) Five major peaks of absorption at 280 nm were pooled separately and their collagen-binding activities were examined, *i.e.*, fraction 1 (△), fraction 2 (□), fraction 3 (▲), fraction 4 (■), and fraction 5 (×), in addition to whole vitronectin (●) and unfractionated heparin-nonbinding fragments (○). (B) The same 5 fractions as in (A) were examined for protein composition by SDS-polyacrylamide gel electrophoresis. Molecular mass (in kDa) is indicated at the left.

and 18 kDa.

DISCUSSION

We have identified several vitronectin fragments which interfere with the binding of ^{125}I-whole vitronectin to immobilized collagen. Vitronectin fragments produced by 70% formic acid were previously mapped by Suzuki *et al.* (30), who concluded that the heparin-nonbinding 40 kDa and 18 kDa fragments are located in the NH_2-terminal half, while the heparin-binding 12–19 kDa fragments are located in the COOH-terminal half of vitronectin. The heparin-nonbinding 40 kDa fragment almost entirely spans the NH_2-terminal half and includes the heparin-nonbinding 18 kDa fragment, but it does not share any region with the heparin-binding 12–19 kDa fragments. Therefore, there should exist two collagen-binding sites (site 1 and site 2) in vitronectin, as shown in a structural model of vitronectin (Fig. 6), in which site 1 may be located near the NH_2-terminus or in the central part of the molecule. Figure 6 also presents a plausible mapping of all the vitronectin fragments referred to in this report. We previously showed that one collagen-binding site is located in the NH_2-terminal half of the molecule on the basis of inhibition analysis of collagen-binding by monoclonal antibodies (31). The NH_2-terminal collagen-binding site identified by use of the monoclonal antibodies seems to correspond to site 1 in this report.

Site 2, reported here for the first time, is located near the heparin-binding region of vitronectin. This site was active in smaller, but not larger, fragments (Fig. 3). Because of the vicinity of site 2 to the heparin-binding site, the larger fragments should contain site 2 according to the mapping by Suzuki *et al.* (30). It is not clear why the collagen-binding activity of site 2 is detected in smaller,

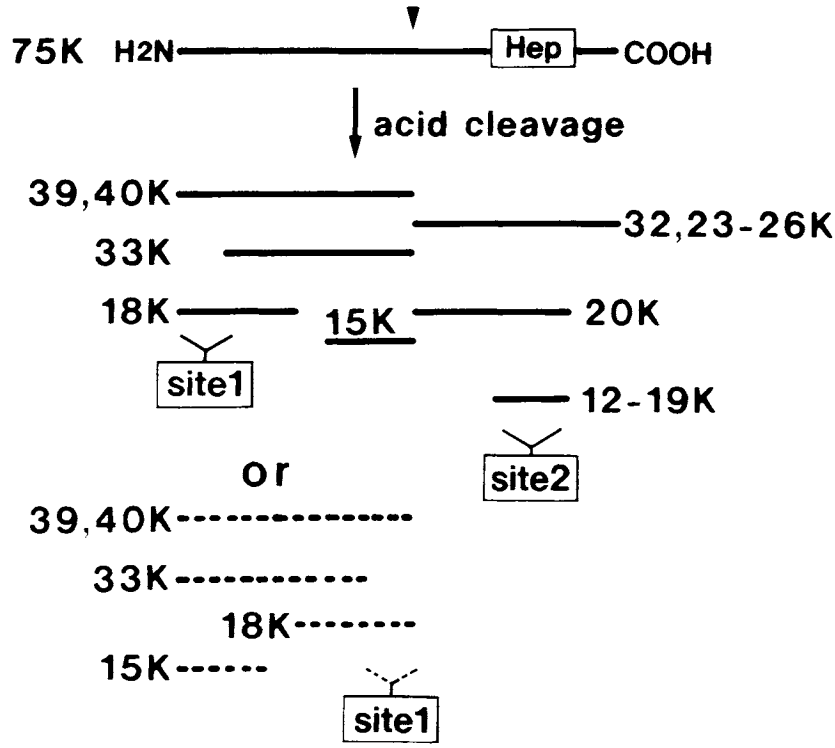

Fig. 6. A plausible mapping of collagen-binding fragments of vitronectin. Vitronectin fragments generated by formic acid in Fig. 1 and collagen-binding fragments identified in Fig. 4 and Fig. 5 are mapped according to the domain model of vitronectin proposed by Suzuki *et al.* (30). Note that there are two possible arrangements in the NH_2-terminal half. Hep indicates heparin-binding site. Site 1 and site 2 indicate two collagen-binding sites.

but not larger, fragments. One possibility is that site 2 is cryptic in the larger fragments but is exposed at the molecular surface in the smaller fragments, by analogy with the conformational conversion of the heparin-binding site. Hϕgåsen *et al.* (32) recently reported that vitronectin spontaneously forms polymers. The polymerization is accelerated by treatment with 8 M urea or ligand-binding. Our purified vitronectin is polymeric (32). The adsorption of vitronectin and vitronectin fragments on collagen may result in some conformational change during the collagen-binding assay used in this report.

Among many collagen-binding proteins, only a few have been analyzed at the amino acid level to elucidate the mechanism of the binding. Roth *et al.* (33) identified two collagen-binding domains of von Willebrand factor and proposed an amino acid sequence VL(K,Q)Y-ID(R,V)P-A(L,H)LL as a common collagen-binding motif. In contrast, Pareti *et al.* (34) isolated two collagen-binding fragments of von Willebrand factor, both of which were different from those identified by Roth *et al.* (33). Takagi *et al.* (35) determined the collagen-binding site of propolypeptide of von Willebrand factor as a decapeptide, WREPSFCALS. Owens and Baralle (36) proposed the 14 amino acid sequence AAHEEICTTNE GVM as the collagen-binding sequence of fibronectin. de Souza and Brentani (37) reported that two peptides, TKKTLRT and SSNTLRS, are responsible for the collagen-binding of fibroblast and polymorphonuclear collagenases, respectively. None of these sequences, however, is present in vitronectin. We have conducted some preliminary experiments to identify the collagen-binding amino acid sequence in vitronectin, using synthetic peptides based on sequences around the heparin-binding site. Surprisingly, a heparin-binding synthetic peptide KKQRFRHRNRKGYRC promoted, but did not inhibit, the binding of ^{125}I-vitronectin to plates bearing immobilized collagen (M. Ishikawa-Sakurai and M. Hayashi, unpublished results).

Deposition of vitronectin on tissues seems to be due to its binding to extracellular matrix macromolecule(s) or cell surface vitronectin receptor. Collagen is a candidate for such an extracellular matrix macromolecule, since purified vitronectin binds to collagen *in vitro*, although most endogenous blood vitronectin does not (25, 26). The activation of the binding can be at least partially understood as a result of conformational change of vitronectin (26).

The binding of vitronectin to collagen is sensitive to

ionic strength (25, 31). The main reasons for the usage of low ionic strength conditions in this study were to obtain higher binding activity, and to allow comparison of the results with data in our previous paper (31). When 0.15 M NaCl was added to the binding solution used in this paper, the amount of vitronectin bound to collagen decreased to only about 10%. This level of binding was reproducible, and vitronectin clearly binds to collagen *in vitro* in such physiological salt solutions, although the physiological meaning of the binding *in vivo* is still uncertain. Collagen is likely to form complexes with proteoglycans *in vivo*, since collagen readily interacts with proteoglycans (38–40). Such complexes may be more effective for vitronectin binding. Many aspects of the interaction between vitronectin and collagen in the extracellular matrix both *in vitro* and *in vivo* still remain uninterpretable at present. Mechanisms of vitronectin deposition on tissues will need to be elucidated if an understanding of the organization of vitronectin *in vivo* is to be reached.

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REFERENCES

1. Preissner, K.T. 1991. Structure and biological role of vitronectin. *Ann. Rev. Cell Biol.,* **7**: 275–310.
2. Tomasini, B.R. and Mosher, D.F. 1991. Vitronectin. *Prog. Hemostasis Thromb.,* **10**: 269–305.
3. Hayashi, M., Hamano, T., Ishikawa-Sakurai, M., and Miyazaki, K. 1993. Vitronectin for functional cell-adhesive biomaterials. In *New Functionality Materials: Design, Preparation and Control* (T. Tsuruta, M. Seno, and M. Doyama, eds.). Elsevier Sci. Pub., Amsterdam, in press.
4. Barnes, D.W. and Silnutzer, J. 1983. Isolation of human serum spreading factor. *J. Biol. Chem.,* **258**: 12548–12552.
5. Hayman, E.G., Engvall, E., A'Hearn, E., Barnes, D., Pierschbacher, D., and Ruoslahti, E. 1982. Cell attachment on replicas of SDS polyacrylamide gels reveals two adhesive plasma proteins. *J. Cell Biol.,* **95**: 20–23.
6. Grabham, P.W., Gallimore, P.H., and Grand, R.J.A. 1992. Vitronectin is the major serum protein essential for NGF-mediated neurite outgrowth from PC12 cells. *Exp. Cell Res.,* **202**: 337–344.
7. Basara, M.L., McCarthy, J.B., Barnes, D.W., and Furcht, L.T. 1985. Stimulation of haptotaxis and migration of tumor cells by serum spreading factor. *Cancer Res.,* **45**: 2487–2494.
8. Hendey, B., Klee, C.B., and Maxfield, F.R. 1992. Inhibition of neutrophil chemokinesis on vitronectin by inhibitors of calcineurin. *Science,* **258**: 296–299.
9. Suzuki, S., Oldberg, A., Hayman, E.G., Pierschbacher, M.D., and Ruoslahti, E. 1985. Complete amino acid sequence of human vitronectin deduced from cDNA. Similarity of cell attachment sites in vitronectin and fibronectin. *EMBO J.,* **4**: 2519–2524.
10. Jenne, D. and Stanley, K.K. 1985. Molecular cloning of S-protein, a link between complement, coagulation and cell-substrate adhesion. *EMBO J.,* **4**: 3153–3157.
11. Pytela, R., Pierschbacher, M.D., and Ruoslahti, E. 1985. A 125/115-kDa cell surface receptor specific for vitronectin interacts with the arginine-glycine-aspartic acid adhesion sequence derived from fibronectin. *Proc. Natl. Acad. Sci. USA,* **82**: 5766–5770.
12. Leavesley, D.I., Ferguson, G.D., Wayner, E.A., and Cheresh, D.A. 1992. Requirement of the integrin $\beta 3$ subunit for carcinoma cell spreading or migration on vitronectin and fibrinogen. *J. Cell Biol.,* **117**: 1101–1107.
13. Seftor, R.E.B., Seftor, E.A., Gehlsen, K.R., Stetler-Stevenson, W.G., Brown, P.D., Ruoslahti, E., and Hendrix, M.J.C. 1992. Role of the $\alpha_V\beta_3$ integrin in human melanoma cell invasion. *Proc. Natl. Acad. Sci. USA,* **89**: 1557–1561.
14. Defilippi, P., Truffa, G., Stefanuto, G., Altruda, F., Silengo, L., and Tarone, G. 1991. Tumor necrosis factor α and interferon γ modulate the expression of the vitronectin receptor (integrin β_3) in human endothelial cells. *J. Biol. Chem.,* **266**: 7638–7645.
15. Koli, K., Lohi, J., Hautanen, A., and Keski-Oja, J. 1991. Enhancement of vitronectin expression in human Hep G2 hepatoma cells by transforming growth factor-β1. *Eur. J. Biochem.,* **199**: 337–345.
16. Hayman, E.G., Pierschbacher, D., Öhgren, Y., and Ruoslahti, E. 1983. Serum spreading factor (vitronectin) is present at the cell surface and in tissues. *Proc. Natl. Acad. Sci. USA,* **80**: 4003–4007.
17. Falk, R.J., Podack, E., Dalmasso, A.P., and Jennette, J.C. 1987. Localization of S protein and its relationship to the membrane attack complex of complement in renal tissue. *Am. J. Pathol.,* **127**: 182–190.
18. Niculescu, F., Rus, H.G., and Vlaicu, R. 1987. Immunohistochemical localization of C5b-9, S-protein, C3d and apolipoprotein B in human arterial tissues with atherosclerosis. *Atherosclerosis,* **65**: 1–11.
19. Dahlbäck, K., Löfberg, H., and Dahlbäck, B. 1986. Localization of vitronectin (S-protein of complement) in normal human skin. *Acta Derm. Venereol.,* **66**: 461–467.
20. Dahlbäck, K., Löfberg, H., and Dahlbäck, B. 1988. Immunohistochemical studies on vitronectin in elastic tissue disorders, cutaneous amyloidosis, Lichen ruber planus and porphyria. *Acta Derm. Venereol.,* **68**: 107–115.
21. Loridon-Rosa, B., Vielh, P., Cuadrado, C., and Burtin, P. 1988. Comparative distribution of fibronectin and vitronectin in human breast and colon carcinomas. An immunofluorescence study. *Am. J. Clin. Pathol.,* **90**: 7–16.
22. Gladson, C.L. and Cheresh, D.A. 1991. Glioblastoma expression of vitronectin and the $\alpha_V\beta_3$ integrin. Adhesion mechanism for transformed glial cells. *J. Clin Invest.,* **88**: 1924–1932.
23. Akama, T., Yamada, K.M., Seno, N., Matsumoto, I., Kono, I., Kashiwagi, H., Funaki, T., and Hayashi, M. 1986. Immunological characterization of human vitronectin and its binding to glycosaminoglycans. *J. Biochem.,* **100**: 1343–1351.
24. Tomasini, B.R. and Mosher, D.F. 1988. Conformational states of vitronectin: preferential expression of an antigenic epitope when vitronectin is covalently and noncovalently complexed with thrombin-antithrombin III or treated with urea. *Blood,* **72**: 903–912.
25. Gebb, C., Hayman, E.G., Engvall, E., and Ruoslahti, E. 1986. Interaction of vitronectin with collagen. *J. Biol. Chem.,* **261**: 16698–16703.

26. Ishikawa, M. and Hayashi, M. 1992. Activation of the collagen-binding of endogenous serum vitronectin by heating, urea and glycosaminoglycans. *Biochim. Biophys. Acta,* **1121**: 173–177.
27. Nagano, Y., Hamano, T., Nakashima, N., Ishikawa, M., Miyazaki, K., and Hayashi, M. 1992. Yolk vitronectin. Purification and differences from its blood homologue in molecular size, heparin binding, collagen binding, and bound carbohydrate. *J. Biol. Chem.,* **267**: 24863–24870.
28. Nakashima, N., Miyazaki, K., Ishikawa, M., Yatohgo, T., Ogawa, H., Uchibori, H., Matsumoto, I., Seno, N., and Hayashi, M. 1992. Vitronectin diversity in evolution but uniformity in ligand binding and size of the core polypeptide. *Biochim. Biophys. Acta,* **1120**: 1–10.
29. Yatohgo, T., Izumi, M., Kashiwagi, H., and Hayashi, M. 1988. Novel purification of vitronectin from human plasma by heparin affinity chromatography. *Cell Struct. Funct.,* **13**: 281–292.
30. Suzuki, S., Pierschbacher, M.D., Hayman, E.G., Nguyen, K., Öhgren, Y., and Ruoslahti, E. 1984. Domain structure of vitronectin. *J. Biol. Chem.,* **259**: 15307–15314.
31. Izumi, M., Shimo-Oka, T., Morishita, N., Ii, I., and Hayashi, M. 1988. Identification of the collagen-binding domain of vitronectin using monoclonal antibodies. *Cell Struct. Funct.,* **13**: 217–225.
32. Hϕgåsen, K., Mollnes, T.E., and Harboe, M. 1992. Heparin-binding properties of vitronectin are linked to complex formation as illustrated by *in vitro* polymerization and binding to the terminal complement complex. *J. Biol. Chem.,* **267**: 23076–23082.
33. Roth, G.J., Titani, K., Hoyer, L.W., and Hickey, M.J. 1986. Localization of binding sites within human von Willebrand factor for monomeric type III collagen. *Biochemistry,* **25**: 8357–8361.
34. Pareti, F.I., Niiya, K., McPherson, J.M., and Ruggeri, Z.M. 1987. Isolation and characterization of two domains of human von Willebrand factor that interact with fibrillar collagen types I and III. *J. Biol. Chem.,* **262**: 13835–13841.
35. Takagi, J., Asai, H., and Saito, Y. 1992. A collagen/gelatin-binding decapeptide derived from bovine propolypeptide of von Willebrand factor. *Biochemistry,* **31**: 8530–8534.
36. Owens, R.J. and Baralle, F.E. 1986. Mapping the collagen-binding site of human fibronectin by expression in *Escherichia coli. EMBO J.,* **5**: 2825–2830.
37. de Souza, S.J. and Brentani, R. 1992. Collagen binding site in collagenase can be determined using the concept of sense-antisense peptide interactions. *J. Biol. Chem.,* **267**: 13763–13767.
38. Mathews, M.B. and Decker, L. 1968. The effect of acid mucopolysaccharides and acid mucopolysaccharide-proteins on fibril formation from collagen solutions. *Biochem. J.,* **109**: 517–526.
39. Scott, J.E. 1980. Collagen-proteoglycan interactions. Localization of proteoglycans in tendon by electron microscopy. *Biochem. J.,* **187**: 887–891.
40. Sorimachi, K. 1992. Inhibition of collagen fiber formation by dextran sulfate in hepatic cells. *Biomedical Res.,* **13**: 75–79.

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