A Novel Synthetic Peptide from the B1 Chain of Laminin with Heparin-binding and Cell Adhesion-promoting Activities

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Abstract. Recent studies using solid-phase-binding assays and electron microscopy suggested the presence of a heparin-binding domain between the inner globule of a lateral short arm and the cross region of laminin. Using the information from the amino acid sequence of the B1 chain of laminin, several peptides were synthesized from areas with a low hydropathy index and a high density of lysines and/or arginines. One of these, peptide F-9 (RYVVLPRPVCFEKGMNYTVR), which is derived from the inner globular domain of the lateral short arm, demonstrated specific binding to heparin. This was tested in direct solid-phase binding assays by coating the peptide either on nitrocellulose or on polystyrene and in indirect competition assays where the peptide was in solution and either laminin or heparin was immobilized on a solid support. The binding of [3H]heparin to peptide F-9 was dramatically reduced when heparin but not other glycosaminoglycans other than heparin (dextran sulfate, dermatan sulfate) were used in competition assays. Modification of the free amino groups of peptide F-9 by acetylation abolished its ability to inhibit the binding of [3H]heparin to laminin on polystyrene surfaces. Peptide F-9 promoted the adhesion of various cell lines (malignoma, fibrosarcoma, glioma, pheochromocytoma) and of aortic endothelial cells. Furthermore, when peptide F-9 was present in solution, it inhibited the adhesion of melanoma cells to laminin-coated substrates. These findings suggest that peptide F-9 defines a novel heparin-binding and cell adhesion-promoting site on laminin.

Laminin is a large (850 kD) basement membrane glycoprotein consisting of three polypeptide chains (B1, B2, A) held together by disulfide bonds (22, 39, 41). By rotary shadowing and electron microscopy, laminin has the shape of an asymmetric cross with three short arms, each with two globular domains near the ends, and one long arm with a large terminal globular domain (6, 22, 39).

Laminin is involved in two general types of interactions. First, it binds to basement membrane macromolecules such as type IV collagen (4, 17), entactin/nidogen (22, 28), heparan sulfate proteoglycan (17), as well as to itself (42). These interactions may be crucial in determining the structure of basement membranes. Second, it has the ability to interact with cell surface molecules, including a 67-kD receptor (19, 21, 38), the CSAT antigen (11), and sulfated glycolipids (29). These interactions with cell surface molecules may influence the adhesion, growth, morphology, and migration (22-24) of various cell types.

Laminin also has the ability to bind to heparin (30). Since macromolecules with heparin-like side chains are present in basement membranes (7, 13, 15, 18, 35) and on cell surfaces (8, 20, 35), this interaction may be very important. Ott et al. (26) have localized a heparin-binding domain on the globule at the distal end of the long arm of laminin. Their studies were performed with laminin fragments derived after various enzymatic digestions. It is possible, however, that other protease-sensitive, heparin-binding sites are present on the laminin molecule. We have recently localized two additional heparin-binding domains on laminin using monoclonal antibodies (34). One domain was localized to the middle of a short arm while the other domain was localized below the intersection of the cross on the long arm of laminin.

In this study, we synthesized various peptides from the published amino acid sequence of the B1 chain (31). We tested these peptides for heparin-binding properties using various solid-phase assays. One of these peptides, F-9, consistently bound specifically to heparin. This peptide was found to promote the adhesion of various cell lines in solid-phase assays and to compete with laminin when added in solution.

Materials and Methods

Reagents

Laminin was isolated from the Engelbreth-Holm-Swarm (EHS) tumor grown subcutaneously in Swiss Webster mice (Simonsen Laboratories, Inc., Gilroy, CA). The mice were made lathyritic by adding 0.1% beta-amino propionitrile to their water, using previously described techniques (4, 14). Heparin (porcine intestinal mucosa, grade I, 15 kD), dextran sulfate (8 kD), and dermatan sulfate (porcine skin, type B, 20 kD) were all purchased from Sigma Chemical Co. (St. Louis, MO).


**Peptide Synthesis and Purification**

Peptides were synthesized on a solid-phase support resin according to the technique of Barany and Merrifield (I). Deprotection and release of peptides was achieved by using HF containing 10% anisole for 1 h at 4°C. Peptides were then extracted with ether, dissolved in 10% aqueous acetic acid, filtered to remove the resin, and lyophilized. Peptides were checked for purity by high pressure liquid chromatography, amino acid analysis, and amino acid sequencing.

Peptides were HPLC purified at a semi-preparative scale using a 21.1 x 250 mm C18 reverse phase column and a gradient of acetonitrile in water containing 0.1% (vol/vol) of trifluoroacetic acid. In the experiments, we originally used HPLC-purified peptides and eventually non-HPLC-purified batches in the cases where the biological activity was tested and found to be similar to the HPLC-purified material.

**Iodination of Laminin and Peptides**

Laminin and peptides (either as shown in Table 1 or with an addition of one tyrosine at their carboxy terminus) were iodinated by using the chloramine T method (12). Laminin was iodinated using chloramine T at a concentration 1,000 times lower than originally described. After iodination, laminin was purified by gel filtration on a 1 x 25 cm Sephacryl G-25 column (Pharmacia Fine Chemicals, Piscataway, NJ) and subsequently dialyzed against Dulbecco's PBS. Iodinated peptides were purified on SEP-PAK C-18 columns (Waters Associates, Milford, MA) and eluted with 50% acetonitrile in water containing 0.1% (vol/vol) trifluoroacetic acid. Eluted peptides were lyophilized and stored at -70°C.

**Modification of Peptide F-9**

The free amino groups of peptide F-9 were modified with acetic anhydride (2). 5 mg of peptide F-9 was dissolved in 0.5 ml DMSO, then 1.5 ml of PBS, pH 7.4, was added. Acetic anhydride at a molar ratio to peptide of 1:51 was added slowly with stirring on ice. The reaction was allowed to proceed for 30 min at 4°C, while the pH was adjusted to 7.0 with 0.1 M NaOH. After the reaction, the modified peptide was separated from the other reagents on a 1 x 25 cm column of Sephadex G-10 (Pharmacia Fine Chemicals) equilibrated with 25 mM NH4HCO3, pH 7.8. Fractions were monitored for peptide content spectrophotometrically at OD280. The eluted protein peaks were lyophilized and stored at -70°C. The total yield was 60% of the starting material.

The extent of modification which occurred at the free ε-amino group of the lysine residue was quantitated by use of the enzyme endoproteinase Lys-C (Boehringer-Mannheim Diagnostics, Inc., Indianapolis, IN). This enzyme cleaves specifically at the carboxy group of any intact lysine; however, when the side chain of the lysine is modified, this cleavage does not take place (37). Control and modified peptide F-9 were incubated for 1 h, at 37°C, at an enzyme to substrate ratio of 1:100. Samples were also incubated in the absence of enzyme. At the end of the incubation, an HPLC profile of the modified peptide was compared to the profile of the control peptide and the reduction of the peptide peak was used as a way to quantify the enzymatic cleavage.

**Cells**

The cells used in this study included: uv-2237-MM murine fibrosarcoma and K-1735-M4 murine melanoma, kindly provided by Dr. J. J. Fidler (M.D. Anderson Hospital, University of Texas Health Science Center, Houston, TX); bovine aortic endothelial cells isolated from bovine carotid thoracic aortas by collagenase digestion were provided by T. Herbst (University of Minnesota, Minneapolis, MN) as described (32); C6 rat glioma cells from the American Type Culture Collection (Rockville, MD) (CCL 107); and PC12 rat pheochromocytoma was kindly provided by Dr. Lloyd Green (New York University, NY). All of the cell lines except the pheochromocytoma cells were maintained in DME (Gibco Laboratories, Grand Island, NY) containing 10% fetal bovine serum (FBS), at 37°C in a humidified incubator containing 5% CO₂. In the case of the pheochromocytoma cells, the media used was DME containing 10% FBS and 5% horse sera.

**Direct Solid-Phase-binding Assays**

The binding of [³H]heparin to the various peptides was measured by direct-binding assays using two different solid supports, nitrocellulose filters and polystyrene plates. For the nitrocellulose dot blot assays, stock solutions of peptides were made at a concentration of 1 mg/ml in 50 mM ammonium bicarbonate, pH 7.8, and serially diluted 1:1 in the same buffer, producing final concentrations ranging from 1 mg/ml to 1 μg/ml. Each concentration was spotted onto a nitrocellulose filter and air dried, using a Minifold Microsample Filtration Manifold (Schleicher & Schuell, Keene, NH). Each well was then rinsed with 200 μl of the same buffer three times. The filter was dried overnight at 22°C, then incubated for 2 h with 50,000 dpm/ml of [³H]heparin (0.3 μCi/μg, New England Nuclear, Boston, MA). The filter was then washed four times, 5 min each, with 10 mM Tris-HCl, pH 7.4, the areas where peptides were spotted were then cut out, immersed in Ecolite scintillation fluid (WestChem, San Diego, CA), and counted in a Beckman LS-3801 scintillation counter.

Direct solid-phase-binding assays were also performed in 96-well poly- styrene Immulon 1 plates (Dynatech Laboratories, Inc., Alexandria, VA) as previously described (34). Stock solutions of peptides at a maximum concentration of 1 mg/ml were prepared in PBS containing 0.02% NaN₃; peptides were then serially diluted 1:1 in the same buffer, producing final concentrations ranging from 1 mg/ml to 2 μg/ml. 50 μl of each concentration was added to the wells and dried overnight at 29°C. Wells were then treated for 2 h at 37°C with 200 μl of 2 mg/ml BSA (Fatty Acid Free, fraction V, ICN Immunobiologicals, Lisle, IL), in 6 mM phosphate, 100 mM NaCl, 60 μM CaCl₂, pH 6.8 (wash buffer) to minimize nonspecific binding. Next, 50 μl of [³H]heparin (10 μg/ml in the same buffer) was added to each well (50,000 dpm/well) and incubated for 2 h at 37°C. The wells were rinsed three times with wash buffer containing 0.1% 3-[3-cholamidopropyldimethylammonio]-1-propanesulfonate and then incubated for 30 min at 60°C with 200 μl of 0.5 N NaOH and 1% SDS. The amount of [³H]heparin bound was quantitated as described above.

**Inhibition-binding Assays**

Inhibition-binding assays were performed by two different techniques: in the first, laminin was immobilized on plastic; in the second, heparin was immobilized on beads.

In the first assay, laminin at 60 μg/ml in PBS containing 0.02% NaN₃ was coated onto 96-well polystyrene plates, using 50 μl per well and dried overnight at 29°C. The wells were then blocked for 2 h at 37°C with 2 mg/ml BSA in wash buffer. Peptides at various dilutions ranging from 0.5 mg/ml to 1 μg/ml in PBS containing 0.1% 3-[3-cholamidopropyldimethylammonio]-1-propanesulfonate were incubated concomitantly with [³H]heparin (25,000 dpm/well; 5 μg/ml final concentration) for 2 h at 37°C in polypropylene tubes. 50 μl of the mixture was then transferred to the laminin-coated wells and allowed to incubate for an additional 2 h at 37°C. The wells were then rinsed with wash buffer containing 0.05% Triton X-100 and [³H]heparin which bound was detected as described above.

In the second assay, heparin-Sepharose beads (Pharmacia Fine Chemicals) were equilibrated in 6 mM phosphate, 100 mM NaCl, 60 μM CaCl₂, pH 6.8. 0.5-ml aliquots of beads (packed beads:buffer at 1:1 volume ratio) were incubated in polypropylene tubes at 37°C for 1 h with each peptide at a final concentration of 1 mg/ml. To each tube, 20 μl of radiolabeled laminin (1 μg/ml; 50,000 dpm/20 μl) was added for another hour at 37°C. At the end of this incubation period, the beads were transferred to small columns, rinsed with 10 μl of buffer (flow rate 2 ml/min), air dried, and placed in the gamma counter to measure the radioactivity left on the column. Nonspecific binding was measured as the amount of [³H]-laminin which bound to plain Sepharose beads. In these experiments, 13.9% of the total laminin counts were found to adhere nonspecifically.

**Specificity of Peptide F-9 Binding to Heparin**

To determine the importance of the heparin structure for this interaction, 3.0 μg per well of peptide F-9 was dried onto 96-well plates as described above. Wells were blocked for 2 h with 2 mg/ml BSA in wash buffer. To each well, 50 μl of [³H]heparin (50,000 dpm/ml) was added concomitantly with various amounts of unlabeled heparin, dextran sulfate, and dermatan sulfate. After incubating for 2 h at 37°C, the wells were washed and the radioactivity was detected as described above.

To determine the importance of the free amino groups of peptide F-9 in its interaction with heparin, we modified peptide F-9 as described above, and then used it in the first type of inhibition assay (laminin-coated plates) exactly as described above.

**Cell Adhesion Assays: Direct Binding**

Five different cell types were tested for their ability to adhere to substrates coated with laminin and the synthetic peptides in assays similar to those previously described (37). Briefly, mid to late log phase cells were incubated...
overnight in DME containing 10% FBS plus either 3 μCi/ml of \([\text{H}]\)thymidine (6.7 Ci/mmol, New England Nuclear, Boston, MA) for the fibrosarcoma and melanoma cell lines, or 30 μCi/ml of \([\text{H}]\)-l-amino acid mixture (240 mCi/ml, ICN Radiochemicals, Irvine, CA) for the glioma and pheochromocytoma cell lines and the endothelial cells. The cells were harvested by gentle trypsinization (two 1-min rinses with 0.05% trypsin and 0.5 mM ethylenediaminetetraacetic acid) and the trypsinization was terminated by the addition of DME containing 10% FBS. Removal of pheochromocytoma cells from the flasks was achieved by incubating cells for 10 min in Hank's balanced salt solution (HBSS) without CaCl2 or MgSO4 (Sigma Chemical Co.), instead of trypsin. All cells were washed with DME containing 20 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid. Adherent cells were solubilized with 200 μl of 0.5 N NaOH and 1% SDS, and quantitated in a scintillation counter.

**Cell Adhesion Assays: Competition of Binding**

The competition assays concentrated on only the melanoma cell line. They were grown, labeled, and harvested as described above. For 30 min before transfer to laminin-coated 96-well plates (coated as described above) and during a 20-min incubation, various potential inhibitors of cell adhesion were present in solution. These included: laminin or peptides F-9, F-11, F-12, or F-13 at a final concentration of 150 μg/ml. Control experiments were done in the absence of inhibitors. Cells were photographed 20 min after they had been transferred to the laminin-coated wells, at a final magnification of 400× with a Nikon DIAPHOT inverted microscope and Panatomic X Film ASA32 (Eastman Kodak, Rochester, NY). All experiments described were performed in triplicate and repeated a minimum of two times.

**Results**

Data obtained by our group in the past using well characterized monoclonal antibodies (33, 34) and direct observations with the technique of rotary shadowing (not shown) strongly suggested that a heparin-binding site on laminin might exist in the area between the inner globule of the lateral short arms and the cross region. To more precisely map this heparin-binding domain, we selected a variety of regions from the published amino acid sequence of the B1 chain of laminin, based on two criteria: First, the region was required to have a relatively low (negative) value of hydropathy index (16), based upon the finding that the heparin-binding domain of vitronectin was localized in a region with the most negative hydropathy index (36). To apply this criterion, we constructed the hydropathy index of the full sequence of the B1 chain, using the Intelligenetics (Mountain View, CA) computer program with a span setting of 7 amino acids (data not shown). Second, since electrostatic forces may play a major role in the interactions between proteins and heparin, the regions selected had a relatively high number of the strongly positively charged amino acid residues lysine and arginine.

The amino acid sequences of the peptides which were synthesized and used in this study and their localization along the B1 chain are shown in Table I and Fig. 1. Other characteristics of each peptide, including the hydropathy value and the number of arginine and lysine residues present are also listed in Table I.

**Binding of \([\text{H}]\)Heparin to Synthetic Peptides**

The direct binding of \([\text{H}]\)heparin to peptides which had been dried onto nitrocellulose filters was determined (Fig. 2). The actual amount of each peptide that was retained on the filter was quantitated by using iodinated peptides, as described in the Materials and Methods. Peptide F-9 was the only peptide to which \([\text{H}]\)heparin bound. Similar experiments using polystyrene plates as a surface to which peptides were dried gave similar results (not shown).

Since these assays were performed with the peptides im-

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**Table I. Peptides Synthesized from the B1 Chain of Laminin**

| Peptide | Amino acid sequence | Residue numbers | Hydropathy index | Number of lysines and arginines |
|---------|---------------------|----------------|-----------------|-------------------------------|
| F-9     | RYVVLPQPVCFEGKMONYTVR | 641-660        | -3.9            | 4                             |
| F-11    | NIDTDTPACDKDGTGRC1K  | 960-978        | -20.7           | 3                             |
| F-12    | VEGVEGPRCDKCTRGY    | 1,133-1,148    | -14.8           | 3                             |
| F-13    | ELTNRTHKFLEKAKALKI  | 1,171-1,188    | -12.9           | 5                             |

* Sasaki et al. (see reference 31).
Figure 2. Binding of heparin to laminin peptides dried onto nitrocellulose. 100 µl of peptide solutions at various concentrations (starting from 500 µg/ml) were blotted on nitrocellulose. Then, the filter was incubated with [3H]heparin for 2 h, as described in Materials and Methods. The actual amount of peptide retained on the filter was calculated in experiments using iodinated peptides with SEM < 5%. F-9 (○), F-11 (●), F-12 (△), and F-13 (□).

mobilized on a solid matrix, we were concerned that immobilization might affect the biological or functional properties of the peptides. Therefore, an inhibition assay was designed in which the binding of [3H]heparin to laminin coated on plastic was monitored in the presence of peptides in solution. Only peptide F-9 was able to inhibit [3H]heparin binding to laminin (Fig. 3); confirming the results of the direct-binding assays. This inhibition could be observed at concentrations as low as 10 µg/ml and reached 50% inhibition at 60 µg/ml. As a control, the [125I]-labeled peptides were added to wells coated with laminin; no binding between laminin and any of the peptides was observed (data not shown). This finding ruled out the possibility that inhibition of heparin–laminin interaction was due to masking of heparin-binding sites on laminin by the peptides.

Since laminin was adsorbed onto plastic in these inhibition assays, it is possible that it was not present in its native state. Therefore, another assay was conducted to further examine the interaction of heparin with peptide F-9 and laminin in an experimental system where both laminin and peptide F-9 would be in solution. Heparin–Sepharose beads were pre-incubated with various peptides at 1 mg/ml, a standard amount of radiolabeled laminin was then added, and the amount of laminin bound was quantitated as described under Materials and Methods. As expected, peptide F-9 was the only peptide which competed for the binding of laminin to heparin (Fig. 4).

The three different experimental approaches described above indicate that peptide F-9 binds to heparin under conditions in which both molecules are in solution or either one of them is immobilized on a solid support.

Specificity of the Interaction Between Peptide F-9 and Heparin

To understand the structural features of peptide F-9 and heparin that are crucial for their interaction, structurally altered molecules were then tested for functional activity. Heparin is composed of a number of negatively charged groups, in particular, sulfate groups. Two other sulfated
polysaccharides with a different structure of the repeated disaccharide unit, dermatan sulfate and dextran sulfate, were tested with heparin in competition-binding experiments. In this assay, peptide F-9 was dried onto polystyrene plates at 3 μg/well. Under these conditions, 150 ng of peptide F-9 is adsorbed and retained on the plastic well. The direct binding of a standard amount of [3H]heparin to the coated peptide was competed off by various concentrations of unlabeled heparin, dermatan sulfate, and dextran sulfate (Fig. 5). A 50% inhibition of binding of [3H]heparin was achieved with ~9 × 10^{-8} M unlabeled heparin, while seven times more dextran sulfate (6 × 10^{-7} M) or 110 times more dermatan sulfate (1 × 10^{-6} M) was required for similar levels of inhibition. These results suggest that although the negative charges of the polysaccharides might play an important role in mediating the binding, other structural features of heparin might have a crucial contribution as well.

Peptide F-9 was then chemically modified to determine whether free amino groups are important for heparin-binding activity. This peptide contains two free amino groups: an α-amino group present on the first arginine residue and a ε-amino group present on the lysine residue. In situ in the BI chain, only the ε-amino group of lysine is present because the α-amino group of arginine is involved in the formation of a peptide bond. The importance of the free amino groups in heparin-binding was determined by modification with acetic anhydride, a chemical which reacts specifically with free amino groups (2, 37).

After treatment with acetic anhydride, the extent of modification of the ε-amino group of lysine was assessed by treatment with endoproteinase Lys-C. This enzyme cleaves at the carboxy terminal of intact lysine residues but fails to do so when the ε-amino group is modified (37). Both intact and modified peptide F-9 were digested as described under Materials and Methods, and the disappearance of the peptide peak on the HPLC profile was measured. In the case of the intact peptide, the peak totally disappeared after 60 min digestion. Under identical conditions, the modified peptide peak was reduced only by 27%. We concluded that over 70% of the ε-amino groups of lysines have been successfully modified.

The ability of modified peptide F-9 to bind heparin in solution was measured using laminin dried onto plastic. As shown in Fig. 3, modified peptide F-9 was no longer able to compete for the binding of heparin to laminin. These results suggest that the free amino groups of peptide F-9 and/or their environment may be crucial areas for the heparin-binding property of this laminin fragment.

**Effect of Peptide F-9 on Cell Adhesion**

Because many cells may exhibit heparin-like structures on their surfaces (8, 20, 25, 35), we investigated the role of our synthetic peptides in the phenomenon of cell adhesion. The results described so far made us put specific emphasis on the activity of peptide F-9.

In the first series of experiments, polystyrene plates were coated with either laminin or peptide F-9. The total amount of each protein dried down was 1 μg. Under these conditions 700 ng of laminin and 69 ng of peptide F-9 remain attached to the solid support during the course of the experiment, as calculated by using radiolabeled proteins. After coating and blocking the plates, radiolabeled cells were incubated in the wells for 2 h, as described in Materials and Methods. The number of cells that adhered in each well was quantitated as a percentage of cell adhesion and was calculated by comparing the radioactivity remaining in the well at the end of the experiment with the total radioactivity added to the well. The results shown in Table II indicate that peptide F-9 promotes the adhesion of all of the cell lines examined at a level comparable to intact laminin. Despite the fact that under our experimental conditions the molarity of the peptide is higher than that of laminin, it is still remarkable that such a small fragment of laminin (less than 1/300th of its molecular mass) is so potent in promoting cell adhesion. Under the same experimental conditions, the other peptides and BSA did not promote or only slightly promoted cell adhesion (data not shown).

We then concentrated on one particular cell line, the melanoma cells, to further study cell adhesion. In this series of experiments, melanoma cells were preincubated with laminin or one of the peptides, then added to laminin-coated wells in the presence of these potential inhibitors. The results shown in Table III demonstrate that laminin was able to inhibit melanoma cell adhesion by 63%, peptide F-9 created a 49% inhibition, whereas the other peptides did not produce any substantial inhibition. These results suggest that peptide F-9 is capable of regulating cell adhesion not only while adsorbed on plastic, but also when present in solution.

The effect of peptide F-9 on the morphology of melanoma cells during adhesion was examined with a photomicroscope during the course of the previously described experiment. As seen in Fig. 6, cells incubated with peptide F-9 in solution were mostly rounded and only a few of them appeared at-

**Table II. Direct Binding of Various Cell Lines to Laminin- and F-9-coated Dishes**

| Cell line               | Laminin* | F-9† |
|-------------------------|----------|------|
| Murine melanoma (M4)    | 28       | 30   |
| Murine fibrosarcoma (MM)| 52       | 64   |
| Rat pheochromocytoma (PC12)| 34   | 60   |
| Rat glioma (C6)         | 50       | 59   |
| Bovine aortic endothelial cells (BAEC)| 18 | 23   |

* Final amount retained on the well: 700 ng.
† Final amount retained on the well: 69 ng.

**Table III. Inhibition of Melanoma Cell Adhesion on Laminin-coated Wells by Various Inhibitors in Solution**

| Competitor* | Inhibition of melanoma cell adhesion |
|-------------|-------------------------------------|
| None        | 0                                   |
| Laminin     | 63                                  |
| F-9         | 49                                  |
| F-11        | 0                                   |
| F-12        | 5                                   |
| F-13        | 9                                   |

* Final concentration: 150 μg/ml.
Figure 6. Inhibition of cell adhesion by peptide F-9 in solution. Melanoma cells were harvested with light trypsinization, washed, and incubated for 30 min in 150 μg/ml of inhibitors (laminin or the various peptides). The cell suspensions were then placed in laminin-coated polystyrene wells at a density of 4 × 10^4 cells/ml, and photographed after 20 min. A is in the absence of inhibitor. The same cell morphology was observed in the presence of peptides F-11, F-12, and F-13. B is in the presence of peptide F-9. The same cell morphology was observed in the presence of laminin.

Discussion

In this study, a synthetic peptide 20 amino acids long from the B1 chain of laminin has been shown to exhibit heparin-binding activity and to promote the adhesion of various cell lines.

Our previous work has demonstrated that, apart from the heparin-binding domain on the globule of the long arm originally described by Ott et al. (26), other heparin-binding domains exist on the laminin molecule (34). One of them was suggested to be localized to the lateral short arm between the inner globule and the cross region (34). Rotary-shadowing observations also supported this finding, by demonstrating a high frequency of heparin binding at or near the inner globule of the lateral short arm (unpublished observations). According to the model of the B1 chain structure by Sasaki et al. (31), peptide F-9 belongs to the amino acid sequences that form the inner globule of the lateral short arm of the B1 chain.

The data in this report suggest that the binding of heparin to peptide F-9 is specific and is not due exclusively to ionic interactions, since other peptides with higher charge densities fail to bind heparin. The factors that determine whether an amino acid sequence will bind to heparin are not fully known. Our findings indicate that neither a very low hydrophathy value nor a high density of positively charged amino acid residues are absolute prerequisites. The peptides synthesized in this study fulfilled both of these criteria, yet only one of them exhibited binding to heparin. Perhaps the exact conformation of each peptide is the crucial factor. The distance that charged amino acid residues are spaced apart may also be an important parameter. Additionally, the structural conformation of the glycosaminoglycan chain might be crucial. The type and amount of hexosamine and uronic acid, the type of bond they form with each other or with the next disaccharide unit (1→3 or 1→4), and the degree of sulfation could all be determining factors in their binding to specific amino acid sequences. These factors could actually confer some specificity in the binding of each type of glycosaminoglycan to different polypeptides.

The finding that modification of the free amino groups of peptide F-9 affects heparin binding suggests that at least two residues (the first arginine and the lysine) and/or their environment might be crucial for this interaction. Further work is in progress in order to understand the minimal structural requirements for the heparin-binding and cell-binding activities of peptide F-9.

In these studies, heparin was used as a prototype for a proteoglycan molecule. Although free heparin may not exist as a bona fide basement membrane component, many heparin-like domains are present in the environment of laminin. These include glycosaminoglycan side chains of basement membrane proteoglycans (7, 13, 15, 18, 35) and of cell surface associated proteoglycans of various cell types associated with basement membranes (8, 20, 35). It has recently been reported that an endothelial cell-derived heparan sulfate pro-
teoglycan may contain certain heparin-like stretches in regions of its side chains (25). Therefore, the heparin-binding sites on laminin may be crucial not only for the structural arrangement of basement membrane components but also for regulating cell adhesion to laminin.

Laminin may contain multiple cell adhesion promoting domains and some of them may be cell-type-specific. Terranova et al. have proposed that a cell-binding site for human breast carcinoma cells exists on a chymotrypsin fragment of laminin that was thought to consist of the inner nonglobular regions of the three short arms (38). Timpl et al. have suggested that proteolytically derived fragments P1, E5, and E6 represent three distinct laminin domains that promote heparan sulfate proteoglycan binding to this laminin domain.

It has been suggested that during development (5) and in certain tissues (14) there are different levels of mRNAs coding for the three laminin chains. If the levels of mRNA reflect the actual amount of each chain present in the tissue and are not due to differential message turnover, then the possibility exists that different forms of laminin could be present. In the kidney, the message for the BI chain has been detected in 13-fold excess of the message for the A chain (15). If laminin is mainly present in this tissue in a form partially or totally lacking the A-chain, then the heparin-binding domain localized on the globule of the long arm will be absent. In this case, the newly described heparin-binding domain on the BI chain of laminin could play a major role in the organization of kidney basement membranes.

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