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Chain-Specific Heparin-Binding Sequences in the Laminin α Chain LG45 Modules[†]

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ABSTRACT: Laminin α chains contain five tandem globular modules (LG1−5) at the C-terminus. Here, we focused on the LG45 module, which play a critical biological role via binding to heparin/heparan sulfate, and examined their chain-specific heparin-binding affinity. The relative heparin-binding affinity of recombinant laminin α chain LG45 proteins was as follows: α5 > α4 > α1 > α2 and α3. The α5 chain LG45 module also promoted the strongest cell attachment. We screened heparin-binding sequences using the recombinant α5LG45 protein and 43 synthetic peptides. Four peptides, A5G71 (GPLPSYLQFVGI) (IC_{50} = 91.8 μM), A5G77 (LVLFLNHGHFVA) (IC_{50} = 7.0 μM), A5G81 (AGQWHRVSVRWG) (IC_{50} = 5.9 μM), and A5G94 (KMPYVSLELEMR) (IC_{50} = 0.84 μM), inhibited the heparin-binding of rec-α5LG45. Additionally, the same four peptides exhibited dose-dependent heparin-binding activity in a solid-phase assay. We found that the α5 chain LG45 module contains four heparin-binding sequences, and this number is higher than that of the other LG45 modules (α2 and α3, one sequence; α1 and α4, two sequences). The data suggest that the active sequences identified from the synthetic peptide screening contribute to the heparin-binding activity of the LG45 module. Most of the heparin-binding sequences in the LG45 modules are located in the N-terminal regions of the LG4 module within the loop regions in the proteins. The data suggest that the N-terminal loop regions of the LG4 module are mainly involved in the heparin/heparan sulfate-mediated biological functions.

Laminins are heterotrimeric basement membrane proteins that have multiple biological functions through interactions with other matrix molecules and cell surface receptors (*1*, *2*). Laminins consist of α, *β*, and *γ* chains that assemble into a triple-stranded coiled-coil structure. At least 16 isoforms of laminins, consisting of five α, three *β*, and three *γ* chains, have been identified (*2*). Laminins have diverse biological activities, including promotion of cell adhesion, cell migration, neurite outgrowth, and tumor metastasis. Five laminin α chains are tissue-specifically and/or developmental stage-specifically expressed. For example, the α1 chain is expressed in the blastocyst neuroectodermally derived tissues and in developing kidney in the early embryo (*3*, *4*). The α2 chain is expressed in both skeletal and cardiac muscle, peripheral nerve, brain, and capillaries (*5*). The α3 chain is mainly localized in skin and in other epithelia (*6*, *7*). The α4 chain is detected in the microvasculature and in smooth muscle (*8*, *9*), and the α5 chain is expressed in multiple tissues during development, in adult microvasculature, and in various epithelia (*10*, *11*). The diversity of the α chain contributes critically to laminin isoform-specific functions (*2*).

The C-terminal globular domain (G domain) of the laminin α chains consists of five laminin G domain-like modules (LG1−5) and plays a critical role in the biological functions. The G domains are cleaved by endogenous proteolytic processing (*12*). The α2 chain G domain is processed in the LG3 module by the furin-type protease (*13*). The cleavage in the α2 chain LG3 module is required for clustering of acetylcholine receptors, contributing to neuromuscular junction formation in concert with agrin (*13*). The linker regions between the LG3 and LG4 modules of the α3 and α4 chains are cleaved, causing the C-terminal LG45 fragments to separate from the laminins. Plasmin promotes the cleavage of the α3 chain and releases the LG45 fragment (*14*). At wound edges in the skin, the leading keratinocytes migrate due to unprocessed laminin-332 containing the α3 chain LG1−5 module. In contrast, quiescent keratinocytes form hemidesmosomes by interaction of the processed laminin-332 releasing the LG45 module via integrins α6*β*4 and α3*β*1 (*15*, *16*). An anti-laminin α4 chain LG45 module antibody cannot detect the LG45 module in heart tissue and has been used to demonstrate that a proteolytic LG45 fragment is released from the heart tissue (*17*). The α1 chain LG45 module exists as a fragment in the ectoplacental cone of the early embryo (*18*). Taken together, proteolytic processing of the G domain regulates laminin functions in a cell- and tissue-type

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manner. Further, most of the α chains are cleaved between LG3 and LG4 in vivo, thus allowing the N-terminal portion, LG1−3 module, to mainly interact with integrins. The C-terminal portion, LG45 module, is mainly involved in heparin binding-related biological functions, including syndecan-mediated adhesion and migration.

We have systematically screened biologically active sequences in laminin molecules using recombinant proteins and a large set of synthetic peptides (*19*−*23*) . We identified various heparin-binding sequences in the LG45 module, including the α1 chain, AG73 (RKRLQVQLSIRT, mouse laminin α1 chain residues 2719−2730) and AG75 (GLIYYVAHQNQM, mouse laminin α1 chain residues 2735−2746) (*24*). AG73 promotes various biological activities and binds syndecan-1, a membrane-associated proteoglycan (*19*, *25*−*28*). In the α2 chain, A2G78 was found to bind heparin and α-dystroglycan (N. Suzuki et al., manuscript submitted for publication). In the α3 chain, A3G75 (KNSFMALYLSKG, human laminin α3 chain residues 1411−1422) was identified as a heparin-binding sequence that promoted syndecan-2- and -4-mediated cell attachment and neurite outgrowth (*29*−*31*). In the α4 chain, A4G82 (TLFLAHGRLVFM, mouse laminin α4 chain residues 1514−1525) exhibited heparin and syndecan-4 binding and cell attachment activity (*32*). In the α5 chain G domain, we identified several cell attachment sequences, including A5G77 (LVLFLNHGHFVA, mouse laminin α5 chain residues 3307−3318) and A5G81 (AGQWHRVSVRWG, mouse laminin α5 chain residues 3337−3348) (*33*, *34*). A5G77 inhibits epithelial branching morphogenesis of mouse submandibular gland ex vivo (*35*). A5G81 promotes syndecan-4-mediated cell attachment and induces tyrosine phosphorylation of focal adhesion kinase (*33*). To date, the heparin-binding sequences of the laminin α5 chain LG45 module have not been fully characterized.

Here, we evaluated heparin-binding of the LG45 modules of α1−α5 chains using five recombinant proteins and found that a α5 chain LG45 recombinant protein had the strongest heparin affinity. We characterized heparin-binding sequences on the α5 chain LG45 module by a systematic peptide screening using rec-α5LG45 and 43 synthetic peptides. We identified four heparin-binding sequences, indicating that the α5 chain LG45 module contains more heparin-binding sequences than the other LG45 modules. The heparin-binding sequences may be important in chain-specific biological activities.

EXPERIMENTAL PROCEDURES

Preparation of Recombinant Laminin α Chain LG45 Proteins. The recombinant proteins of the mouse laminin α1 (*24*), α2 (N. Suzuki et al., manuscript submitted for publication), α3 (*36*), α4 (*32*), or α5 chain LG45 (*33*) modules with the human IgG Fc portion were prepared as previously described. The PCR primers with restriction enzyme sites (forward primers, *Avr*II; reverse primers, *Avr*II for α1 and α4 and *Bam*HI for α2, α3, and α5) were as follows: nucleotides 8158−9297 of the laminin α1 chain, 5′-GAGCCTAGGGCTACCACAG-CCTGAACTG-3′ (forward) and 5′-GAGCCTAGGCC-CTCAGGCCCGGGGCAGGAATG-3′ (reverse); nucleotides 8158−9372 of the laminin α2 chain, 5′-GAGCCTAGGG-ACCATGGTGCATGGCCCTTG-3′ (forward) and 5′-GA-GGGATCCCCGTTCCAGGGCCTTGGCAAAATTAACC-3′ (reverse); nucleotides 4099−5196 of the laminin α3 chain, 5′-GAGCCTAGGGTGGCAAGATGGGAAGTCC-3′ (forward) and 5′-GAGGGATCCCCGTGGTCAGGGCAGCCATTCA-G-3′ (reverse); nucleotides 4538−5656 of the laminin α4 chain, 5′-GAGCCTAGGGGCTCCAAGAGATTCCCAC-3′ (forward) and 5′-GAGCCTAGGCCGGCTGTGGGACAG-GAGTTGATG-3′ (reverse); and nucleotides 9703−10905 of the laminin α5 chain, 5′-GAGCCAGGGCAACCCAGCCAG-GACCTTG-3′ (forward) and 5′-GAGGGATCCCCATGC-CAAAGTAGCGGGGAG-3′ (reverse). The recombinant α chain LG45 modules (rec-α1LG45 to rec-α5LG45) were expressed in 293T cells by transfection using Lipofectamine 2000 (Invitrogen, Carlsbad, CA). These proteins were purified from the conditioned medium using a Protein A-Sepharose column (GE Healthcare Bio-Sciences Corp, Piscataway, NJ) and a heparin-Sepharose column (GE Healthcare Bio-Sciences Corp.). The eluted fraction was dialyzed with 20 mM Tris-HCl (pH 7.5) containing 150 mM NaCl. Protein concentrations were determined with the BCA assay kit (Thermo Fisher Scientific Inc., Rockford, IL).

Synthetic Peptides. All peptides were synthesized manually using the 9-fluorenylmethoxycarbonyl (Fmoc)-based solid-phase strategy and prepared in the C-terminal amide form as previously described (*33*). Amino acid derivatives and resins were purchased from Novabiochem (La Jolla, CA). The respective amino acids were condensed manually in a stepwise manner using 4-(2′,4′-dimethoxyphenyl-Fmoc-aminomethyl)phenoxy resin. Dimethylformamide (DMF) was used during the synthesis as a solvent. For condensation, diisopropylcarbodiimide/*N*-hydroxybenzotriazole was employed, and for deprotection of N^{α}-Fmoc groups, 20% piperidine in DMF was employed. The resulting protected peptide resins were deprotected and cleaved from the resin using a trifluoroacetic acid/thioanisole/*m*-cresol/ethanedithiol/H_2O mixture (80:5:5:5:5, v/v) at 20 °C for 3 h. The crude peptides were precipitated and washed with diethyl ether and then purified by reverse-phase high-performance liquid chromatography (HPLC) using a C_{18} column with a water/acetonitrile gradient containing 0.1% trifluoroacetic acid. The purity and identity of the peptides were confirmed by analytical HPLC and by an electrospray ionization mass spectrometer at the Central Analysis Center of the Tokyo University of Pharmacy and Life Sciences.

Effect of NaCl on the Heparin-Binding to Various α Chain LG45 Recombinant Proteins. The effect of NaCl on heparin-binding to the five LG45 recombinant proteins (rec-LG45) was examined. rec-LG45s (5 μg) and heparin-Sepharose (1 mg) were mixed in 40 μL of 10 mM Tris-HCl (pH7.4) and incubated for 1 h. The beads were pelleted by centrifugation and washed twice with binding buffer. The proteins bound to heparin-Sepharose were eluted with 40 μL of binding buffer, including 100−800 mM NaCl by stepwise elution. Each eluate was analyzed by Western blotting (8% SDS−PAGE under reducing conditions) using biotinylated goat anti-human IgG Fc (1:1000 dilution) and a streptavidin-conjugated horseradish peroxidase (SA−HRP) (Sigma, St. Louis, MO) (1:2000 dilution) using an ECL kit (GE Healthcare Bio-Sciences Corp.).

Peptide Inhibition of Binding of Heparin to rec-α5LG45. The effect of peptides on the heparin-binding of rec-α5LG45 was tested using heparin-Sepharose beads as previously described (*24*) with some modifications. The rec-α5LG45 protein (3 μg), heparin-Sepharose beads (1 mg, GE Healthcare Bio-Sciences Corp.), and peptide (20 μg) were mixed in 70 μL of 10 mM Tris-HCl (pH 7.4), containing 100 mM NaCl (binding buffer). After a 1 h incubation, the beads were pelleted by centrifugation. The supernate was removed, and the beads were washed twice with binding buffer. The rec-α5LG45 protein bound to the beads was

extracted with SDS−PAGE sample buffer, analyzed by 8% SDS−PAGE under reducing conditions, and detected by Western blotting using biotinylated goat anti-human IgG Fc (Jackson ImmunoResearch Laboratories, West Grove, PA) (1:1000 dilution) and SA−HRP (1:2000 dilution) using an ECL kit.

Solid-Phase Heparin-Binding Assay Using Peptide-Coated Plates. A heparin-binding assay with peptide-coated plates was conducted using biotinylated heparin (Celsus Laboratories Inc., Cincinnati, OH) as previously described (N. Suzuki et al., manuscript submitted for publication) with some modifications. Various amounts of peptides in Milli-Q water (50 μL) were coated onto ninety-six-well ELISA plates (AGC Techno Glass, Chiba, Japan) and dried overnight at room temperature. The wells were washed with 0.05% Tween 20 in PBS (washing buffer) and then blocked with 3% bovine serum albumin (BSA) (Sigma) in PBS at room temperature for 2 h. After the sample had been washed, 10 ng of biotinylated heparin was added to the wells and incubated at 37 °C for 1 h. Then, 10 ng of streptavidin-conjugated horseradish peroxidase (Sigma) was added to the wells and incubated at 37 °C for 1 h. The bound protein was detected by TMB solution (Sigma) at 450 nm absorbance using a model 550 Microplate Reader (Bio-Rad Laboratories, Hercules, CA).

Cell Attachment Assay. Ninety-six-well plates (Nalge Nunc, Rochester, NY) were coated with five rec-LG45s (2 μg/well) and prepared as previously described (*33*) with some modifications. HT-1080 human fibrosarcoma cells (3×10^3 cells/well) were added to the wells and incubated for 120 min at 37 °C in a humidified atmosphere of 5% CO_2. After being washed with prewarmed PBS containing calcium and magnesium, cells were fixed and stained with a 0.2% crystal violet aqueous solution in 20% methanol for 10 min at room temperature. After the wells were washed with distilled water (two times), the attached cells were counted in three different fields using a BZ-8000 microscope (Keyence, Osaka, Japan).

RESULTS

Heparin-Binding Affinity of Laminin α Chain LG45 Proteins. We prepared five recombinant laminin α chain LG45 proteins (rec-α1LG45−rec-α5LG45) using the 293T cells as described previously (*24*, *31*, *32*) (N. Suzuki et al., manuscript submitted for publication) (Figure 1A). The proteins correspond to the mouse laminin sequences except for the α3 chain. Since mouse rec-α3LG45 is rapidly cleaved during the expression and purification process, we used human rec-α3LG45 instead of mouse protein in this study (84% homology vs mouse).

We examined the heparin-binding affinity of the recombinant proteins using heparin-Sepharose beads (Figure 1B). The proteins were mixed with heparin-Sepharose beads and washed with various concentrations of NaCl (from 100 to 800 mM). Then, the eluted protein was analyzed by SDS−PAGE (Figure 1B). The proteins were eluted with various NaCl concentrations: 600 mM for rec-α5LG45, 400 mM for rec-α4LG45, 300 mM for rec-α1LG45, and 200 mM for rec-α2LG45. rec-α3LG45 exhibited a broad elution pattern (from 100 to 800 mM NaCl). These results indicate that rec-α5LG45 has the highest heparin-binding affinity.

Effect of Peptides on the Heparin-Binding of the rec-α5LG45 Protein. Previously, we identified heparin-binding sequences in the LG45 module of the laminin α1−α4 chains using a systematic peptide screening method with a recombinant

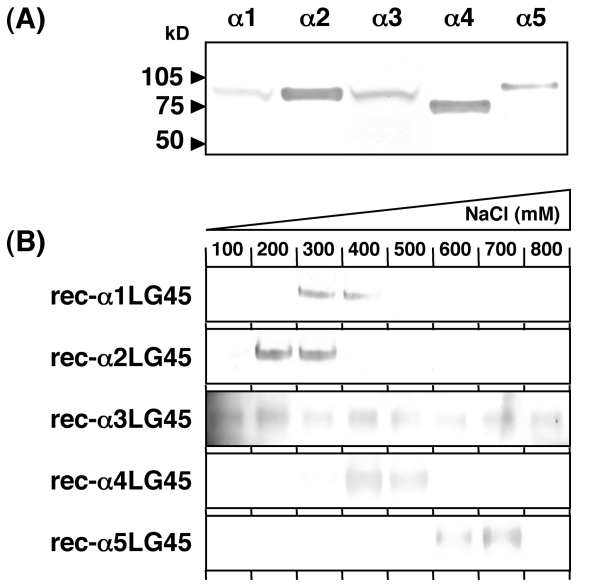

FIGURE 1: Heparin-binding activity of recombinant LG45 proteins. (A) Five recombinant LG45 proteins were expressed in 293T cells as previously described (*24*, *31*, *32*) (N. Suzuki et al., manuscript submitted for publication). The purity of proteins was analyzed via SDS−PAGE, followed by protein staining. (B) The rec-LG45s and heparin-Sepharose beads were mixed and incubated. The proteins bound to heparin-Sepharose were eluted with NaCl (from 0 to 800 mM). Duplicate experiments gave similar results.

protein and synthetic peptides (*31*, *32*, *38*) (N. Suzuki et al., manuscript submitted for publication). Here, we focused on the α5 chain LG45 module and screened heparin-binding sequences using the systematic peptide screening method with rec-α5LG45 and synthetic peptides. Forty-six overlapping peptides covering the laminin α5 chain LG45 module were prepared (Figure 2). The effect of the 43 soluble peptides on the interaction between rec-α5LG45 and the heparin-Sepharose beads was evaluated (Figure 2). Four peptides, A5G71 (GPLPSYLQFVGI), A5G77 (LVLFLNHGHFVA), A5G81 (AGQWHRVSVRWG), and A5G94 (KMPYVSLELEMR), significantly inhibited the heparin-binding of rec-α5LG45, while rest of the soluble peptides did not (Figure 2). Scrambled peptides of A5G71, A5G77, A5G81, and A5G94, A5G71S (YLGQPLVPSIGF), A5G77S (FLHLVNAFGHLV), A5G81S (WARGHQVRVGWS), and A5G94S (MSPYLEKLMERV), respectively, were also prepared and found to be inactive (Figure 2). These results indicate that the inhibitory effect of A5G71, A5G77, A5G81, and A5G94 on the heparin-binding of rec-α5LG45 is sequence-specific.

Next, we examined their inhibitory effects on the heparin-binding of rec-α5LG45 using various amounts of the four active peptides (Figure 3). These four active peptides exhibited a dose-dependent inhibitory effect with the following IC_{50} values: 91.8 μM (A5G71), 7.0 μM (A5G77), 5.9 μM (A5G81), and 0.84 μM (A5G94) (Figure 3). These results suggest that the four sequences are important for heparin-binding in the α5 chain LG45 module.

Heparin-Binding Activity of Peptides. We next examined the direct heparin-binding activity of the peptides using peptide-coated plates. In the solid-phase binding assay, biotinylated heparin was added to the peptide-coated plates. A5G77, A5G71, and A5G94 exhibited strong heparin-binding activity in a dose-dependent manner (Figure 4). A5G81 exhibited weak heparin-binding activity (Figure 4). In contrast, the scrambled peptides, A5G71S, A5G77S, A5G81S, and A5G94S, did not

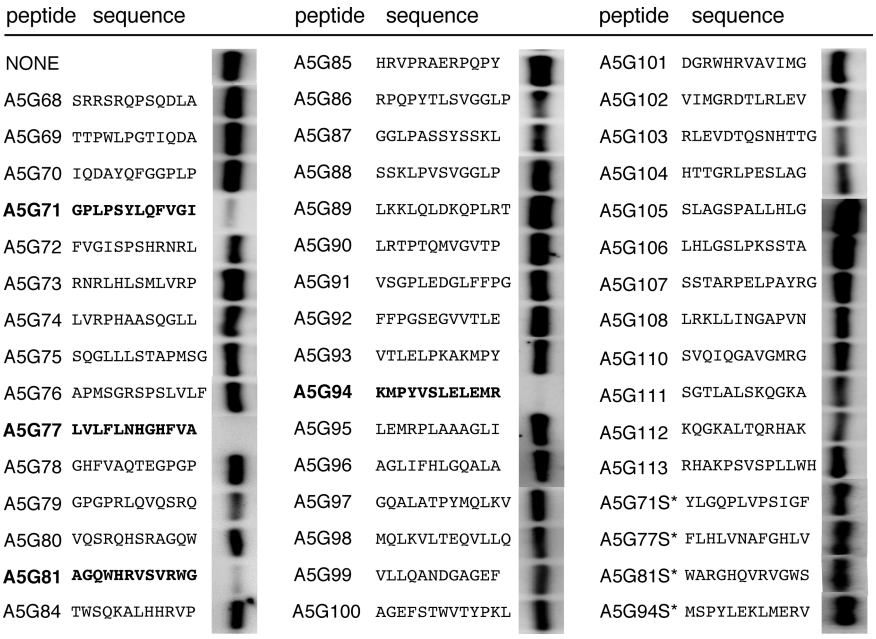

FIGURE 2: Effect of peptides on rec-α5LG45 protein−heparin binding. Sequences were derived from the mouse laminin α5 chain G domain (positions 3313−3718). Heparin-Sepharose beads (1 mg), peptide (20 μg), and the rec-α5LG45 protein (3 μg) were incubated in 70 μL of 10 mM Tris buffer (pH 7.4) at 4 °C for 1 h. After the beads were washed, rec-α5LG45 bound to the heparin-Sepharose beads was analyzed by Western blotting. Active peptides are shown in bold. Triplicate experiments gave similar results. Asterisks denote scrambled peptides of the four active peptides.

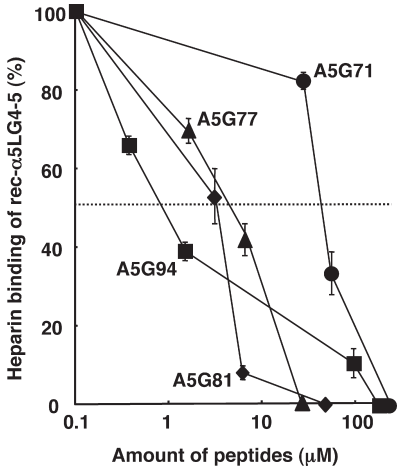

FIGURE 3: Inhibitory effect of peptides on binding of rec-α5LG45 to the heparin. The heparin-binding of rec-α5LG45 was inhibited by various amounts of peptides, and the IC_{50} value of each peptide was examined. The relative amount (percent) of rec-a5LG45 bound to the heparin-Sepharose bead was examined by SDS−PAGE and assessed by NIH image 1.63. IC_{50} values of A5G71, A5G77, A5G81, and A5G94 were 91.8, 7.0, 5.9, and 0.84 μM, respectively. Triplicate experiments gave similar results.

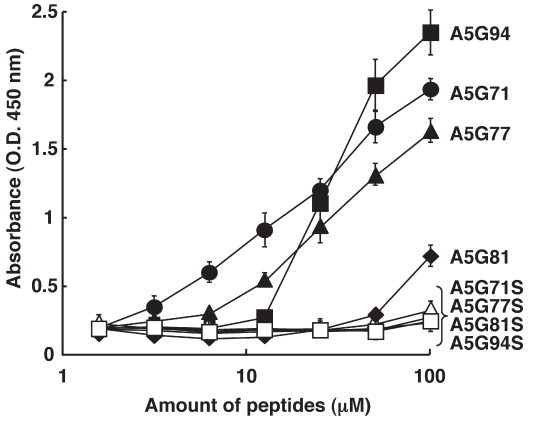

FIGURE 4: Binding of biotinylated heparin to peptide-coated plates. Ninety-six-well plates were coated with various amounts of peptides. After the plates had been blocked with BSA, biotinylated heparin (10 ng/75 μL) was added and incubated for 1 h. Then the biotinylated heparin bound to the peptides was detected by streptavidin-conjugated horseradish peroxidase. Triplicate experiments gave similar results.

exhibit activity in this assay (Figure 4). These results firther suggest that the heparin-binding activity of the peptides is sequence-specific.

We identified four heparin-binding sequences in the α5 chain LG45 module by systematic peptide screening using a recombinant protein and a set of synthetic peptides. Previously, we identified heparin-binding sequences in the LG45 modules of the α1−α4 chains using similar methods. The α5 chain LG45 module contains a higher number of heparin-binding sequences compared with that of the other LG45 modules (α2 and α3 chains, one sequence; α1 and α4 chains, two sequences). To evaluate the relation between the number of heparin-binding sequences and biological function, we examined the cell attachment activity of the recombinant LG45 proteins.

Cell Attachment Activity of the Proteins. The cell attachment activity of the five recombinant proteins was examined using HT-1080 human fibrosarcoma cells. The cells were added to the protein-coated plates, and after a 1 h incubation, attached cells were counted (Figure 5). rec-α1LG45 and rec-α5LG45 promoted strong cell attachment, and rec-α2LG45, rec-α3LG45, and rec-α4LG45 exhibited weak activity. These results suggest that heparin-binding activity contributes to cell binding and that the number of heparin-binding sequences correlates with biological function.

DISCUSSION

Here, we focused on the functionally important laminin α chain LG45 modules and determined their chain-specific

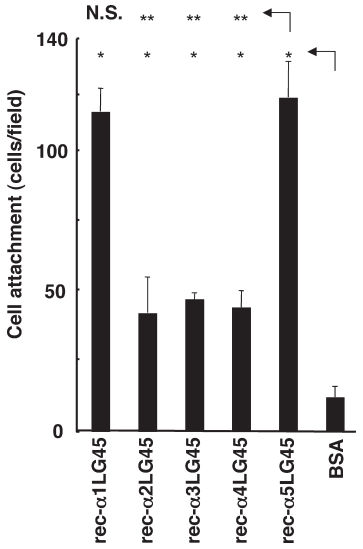

FIGURE 5: Cell attachment activity of the recombinant α chain LG45 proteins. The proteins (2 μg/well) were coated on the 96 well plates for 1 h at room temperature. HT1080 cells (3000 cells/well) were added and incubated for 1 h. Then attached cells were counted. Duplicate experiments gave similar results. Each value represents the mean of three separate determinations ± the standard deviation. Statistical analyses were performed using an unpaired Student's t test. One asterisk denotes a p value of <0.05, and two asterisks denote a p value of <0.005. N.S. means not significant.

chain	peptide	sequence
α1	AG73	R K R L Q V Q L S I R T
α1	AG75	G L I Y Y V A H Q N Q M
α2	A2G78	G L L F Y M A R I N H A
α3	A3G75	K N S F M A L Y L S K G
α4	A4G82	T L F L A H G R L V F M
α4	A4G83	L V F M F N V G H K K L
α5	A5G71	G P L P S Y L Q F V G I
α5	A5G77	L V L F L N H G H F V A
α5	A5G81	A G Q W H R V S V R W G
α5	A5G94	K M P Y V S L E L E M R

FIGURE 6: List of heparin-binding peptides from the laminin α chain LG45 modules. Heparin-binding sequences identified here and previously reported are listed (*24*, *31*, *32*) (N. Suzuki et al., manuscript submitted for publication). Basic amino acids are inside black squares. Aromatic amino acids are inside white hexagons. His residues are inside gray hexagons.

heparin-binding activity using recombinant proteins. The relative heparin-binding affinities of the proteins were as follows: rec-α5LG45 > rec-α4LG45 > rec-α1LG45 > rec-α2LG45 and rec-α3LG45. Previously, we identified two heparin-binding peptides from the α1LG45 and α4LG45 modules and one peptide from the α2LG45 and α3LG45 modules (*24*, *31*, *32*) (N. Suzuki et al., manuscript submitted for publication). Here, four heparin-binding peptides were identified from the α5 chain LG45 module and are likely responsible for the strongest heparin binding affinity among the laminin LG45 modules. rec-α1LG45, which contains only one heparin-binding sequence, promoted strong cell attachment similar to that of rec-α5LG45. We previously identified two active peptides from the mouse laminin α1LG4 module, AG73 for heparin/heparan sulfate binding and EF-1 (DYATLQLQEGRLHFMFDLG, mouse laminin α1 chain residues 2747−2765) for integrin α2β1 binding (*24*, *37*). The AG73 and EF-1 sequences cooperate and induce cell adhesion through the synergetic interaction of syndecan and integrin α2β1 (*38*). These results suggest that the strong cell attachment activity of rec-α1LG45 was promoted by not only heparin-binding but also integrin α2β1 binding. Thus, the α5 chain LG45 module contains more heparin-binding sequence than the other α chain LG45 modules and is important in heparan sulfate proteoglycan-mediated biological functions.

Heparin-binding sequences in the α chain LG45 modules identified here and previously are listed (Figure 6). Most of the peptides contained basic amino acids, Arg, Lys, and His, except for A5G71. The basic amino acids are important for binding to acidic groups in the heparin. Additionally, most of the peptides contained aromatic amino acids, Tyr, Phe, Trp, and His, except for AG73, and β-branched amino acids, Ile and Val, except for A3G75. These hydrophobic amino acid groups, aromatic and β-branched amino acids, may contribute to the inter- or intramolecule interactions of the peptide and may provide the basic amino acid cluster for heparin-binding. At this stage, no clear consensus sequences were observed. With these data taken together, we conclude that ionic, hydrophobic, and electrostatic interactions may be important for the heparin-binding activity of peptides.

We aligned amino acid sequences of the α chain LG45 modules on the basis of the previous report (*12*, *39*) and identified the locations of the 10 heparin-binding sequences (Figure 7). Three heparin-binding sequences are localized in the N-terminal region of the laminin α5 chain LG4 module (Figure 7). The heparin-binding sequences in the α1−α4 chain LG4 modules are also located in the N-terminal region (Figure 7). The N-terminal part of the LG4 module may play a critical role in the interaction with heparin/heparan sulfate proteoglycans. The different localization of these sites may provide a rationale for chain-specific biological function and/or for the cell- and tissue-type specific activity. These heparin-binding peptides are useful for elucidating the mechanism of the laminin chain-specific biological activities.

The LG modules consist of a 14-stranded β-sheet (A−N) sandwich structure (*39*) (Figure 7). A5G77 as well as A3G75 and A4G82 are located in the connecting loop regions between β-strands and exposed on the protein surface in the LG4 module as described previously (*12*). A5G71 and A5G81 are also in the loop regions of the B and C strands and the G and H strands, respectively. The A5G94 sequence is a homologous region of AG73, the strongest heparin-binding peptide (*24*), and is in the loop regions of the B and C strands. All the heparin-binding peptides previously identified in the other LG4 modules are also located in the loop regions. The loop regions in the LG modules may be located at the molecular surface and interact with heparin/heparan sulfate proteoglycans. We previously described that AG73, A3G75, and A4G82 interact with syndecans, membrane-associated proteoglycans (*26*, *31*, *32*). A5G71, A5G77, A5G81, and A5G94 may interact with syndecans, but this has not been demonstrated. Previously, we cyclized biologically active peptides in loop regions to examine the structural requirements (*37*, *40*, *41*). The cyclic peptides enhanced the biological activity, suggesting that the loop structure of the peptides is important for biological activities. Cyclic peptides of the α5 chain

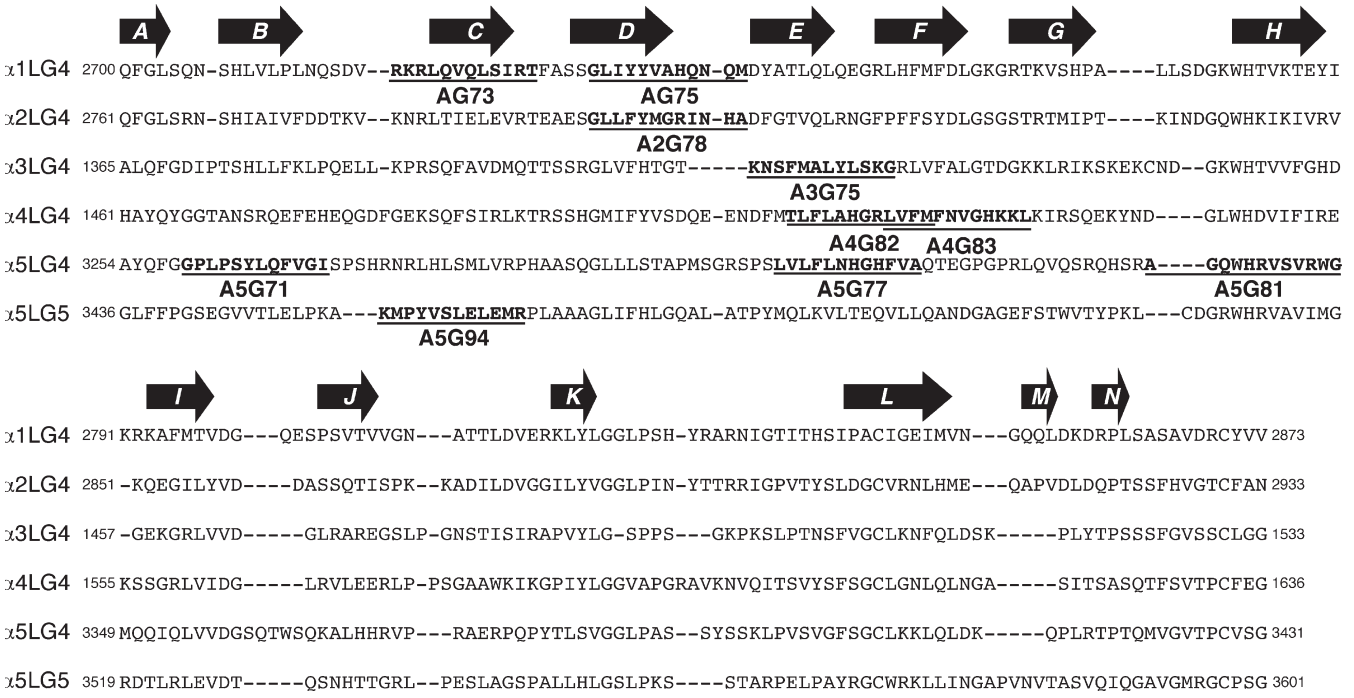

FIGURE 7: Active peptides and amino acid sequence alignment of the laminin α chain LG4 modules. The positions of active peptides are mapped on the structure-based sequence alignment of LG modules of the α chain (*12*, *39*). β-Strands are denoted with black arrows. The α1, α2, α4, and α5 chains are mouse laminin sequences (*12*, *39*). The α3 chain is the human sequence (*31*). Sequences of the α5 chain LG5 module examined in this paper are also indicated. Heparin-binding sequences are underlined.

heparin-binding peptides have the potential to enhance the activity.

In this paper, we identified four heparin-binding sequences in the laminin α5 chain LG45 module. These heparin-binding peptides are useful in evaluating the biological functions of the laminin α5 chain. We also demonstrated that the laminin α5 chain LG45 module has the highest heparin-binding affinity compared with those of the other α chain LG45 modules. The laminin α5 chain may interact with heparin and/or heparan sulfate in vivo. These heparin-binding sequences as well as differences in the heparin-binding affinity of LG45 modules may contribute and regulate the chain-specific biological activities of laminins.

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