An Essential Heparin-Binding Domain in the Fibroblast Growth Factor Receptor Kinase

Mikio Kan, Fen Wang, Jianming Xu, John W. Crabb, Jinzhao Hou, Wallace L. McKeehan*

Heparin or heparin-like heparan sulfate proteoglycans are obligatory for activity of the heparin-binding fibroblast growth factor (FGF) family. Heparin interacts independently of FGF ligand with a specific sequence (K18K) in one of the immunoglobulin-like loops in the extracellular domain of the FGF receptor tyrosine kinase transmembrane glycoprotein. A synthetic peptide corresponding to K18K inhibited heparin and heparin-dependent FGF binding to the receptor. K18K and an antibody to K18K were antagonists of FGF-stimulated cell growth. Point mutations of lysine residues in the K18K sequence abrogated both heparin- and ligand-binding activities of the receptor kinase. The results indicate that the FGF receptor is a ternary complex of heparan sulfate proteoglycan, tyrosine kinase transmembrane glycoprotein, and ligand.

Matrix or transmembrane heparan sulfate proteoglycans have been viewed as a low-affinity reservoir of FGF binding sites distinct from the signal-generating transmembrane receptor. Studies have shown that FGFs interact with heparin-like glycosaminoglycans independently of other proteins, heparin protects FGFs from heat and proteolytic inactivation, and heparin increases the affinity of FGF for cell surface receptors (*1*). In this report, we show that heparin and cell-derived heparan sulfate proteoglycans interact independently of FGF ligand with a specific domain in the NH_2-terminus of immunoglobulin (Ig)-like loop II of the transmembrane tyrosine kinase glycoprotein component of the FGF receptor (FGF-R) complex (*2*). The intact structural domain and associated heparin or heparan sulfate proteoglycan is obligatory for binding of FGF.

Exposure of cells to 0.6 M NaCl before or after ligand-binding assays had no effect on ^{125}I-labeled binding of FGF to cell-surface receptors (Fig. 1A, inset); however, increased salt concentrations during binding assays decreased the binding of FGF relative to that of epidermal growth factor (EGF) (Fig. 1A). This indicated that the binding of FGF to receptors was disrupted by concentrations of salt (<1 M) insufficient to inhibit the association of FGF with heparin (*1*). Immunopurified receptor kinase, FGF-R1β1, derived from insect cells infected with recombinant baculovirus (3), bound ^{125}I-labeled FGF in the presence of 0.15 M NaCl without the addition of heparin (Fig. 1B). However, washing the complex with 0.5 M NaCl prevented binding of FGF. Exposure of the receptor to heparin restored ligand-binding activity even after the receptor complex was removed from the solution containing heparin before the binding. This suggested that the endogenous salt-extractable cofactor was heparin or a heparin-like molecule. Similar to heparin, nonreceptor heparin-like FGF-binding molecules that were released from cells by mild trypsin treatment (*4*) also restored ligand-binding activity to the salt-washed, inactive recombinant receptor kinase. The salt-labile association of FGF-R with immobilized heparin in the absence of FGF confirmed that the FGF-R kinase interacts with heparin independently of ligand.

To identify the heparin-binding domain, we immobilized recombinant 105-kD FGF-R1β1 on heparin-agarose and subjected it to mild trypsin treatment. Heparin protected a fragment of about 30 kD (the 45-kD band includes the 15-kD ligand) (Fig. 2A). Purification of the heparin-binding, trypsin-resistant fragments by chromatography on copper-chelating agarose and reversed-phase high-performance liquid chromatography (HPLC) revealed 33- and 30-kD species with NH_2-terminal sequences P^{23}SPTLPEQ and E^{51}TDNTKPN, respectively (Fig. 2B) (*5*). Residue P^{23} is two residues past the NH_2-terminal A^{21} residue of mature FGF-R1β1 (*3*), and the E^{51} residue is ten residues upstream of the beginning of loop II at the COOH-terminus of a characteristic cluster of acidic residues referred to as the acidic box. Failure of the 30-kD fragment to react with antibody D26K (*3*) to FGF-R1β1 residues D^{31} to K^{56} containing the acidic box (Fig. 2C) confirmed that the 33- and 30-kD fragments differ in the absence or presence of residues P^{23} to K^{50}. Reactivity of the 33- and 30-kD species with antibody H27V (*3*) to the 18 COOH-terminal residues of the sequence H^{152} to V^{178} (Fig. 2C) suggested that the heparin-protected fragments spanned at least Ig loop II and the sequence between loop II and loop III. Failure to react with antibody L30K (*3*, *6*) to residues E^{233} to Y^{250} (Fig.

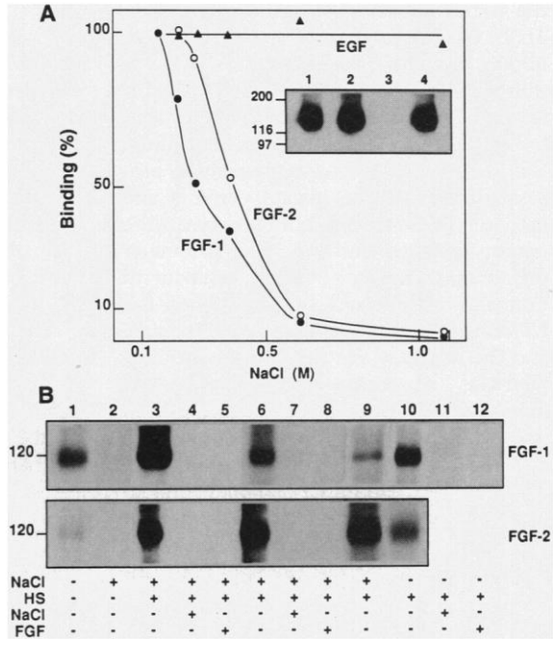

Fig. 1. Requirement for a heparin-like component in the FGF-R complex. (**A**) Inhibition of FGF binding to cells by NaCl. Specific binding of ^{125}I-labeled FGF-1, FGF-2, or EGF to HepG2 cells was determined as described (*12*). Specifically bound factor is expressed as a percentage of that bound in the presence of 0.15 NaCl in phosphate-buffered saline (PBS). The indicated concentrations of NaCl were present during the binding assays. (**Inset**) Iodine^{125}-labeled FGF-1 receptor complexes from HepG2 cells washed with PBS (lane 1); cells washed with 0.6 M NaCl in PBS before (lane 2) or after (lane 4) binding assays or from cells with 0.6 M NaCl in PBS in the binding assays (lane 3). Receptor complexes were covalently cross-linked with 1 mM disuccinimydyl suberate (DSS) and analyzed by 7.5% SDS–polyacrylamide gel electrophoresis (PAGE) and autoradiography (*12*). Molecular size markers are indicated at the left (in kilodaltons). (**B**) Heparin requirement for FGF binding to recombinant FGF-R1β (*13*). Soluble heparin (2 μg/ml; 170 U/mg) was the HS component in the binding assays in lanes 3 to 8. In lanes 6 to 8, beads were incubated with heparin (2 μg/ml) and then washed with PBS before the binding assays. The salt-washed immunocomplex in lane 9 was incubated with 10 μl of the material (HS) released from the surface of HepG2 cells by trypsin (*4*). Lanes 10 to 12 show the binding of ^{125}I-labeled FGF to 10 μl of heparin-agarose (HS) beads after incubation with 20 μl of infected Sf9 cell lysate.

W. Alton Jones Cell Science Center, Inc., Old Barn Road, Lake Placid, NY 12946.

*To whom correspondence should be addressed.

2C) showed that the protected fragments were truncated before or within the sequence in loop III. Treatment with N-glycosidase F suggested that the 33- and 30-kD fragments contained about 50% (13 to 14 kD) (Fig. 2D, lane 1) of the 25 to 30 kD of carbohydrate in the parent recombinant FGF-R1β1 (*3*). Cleavage of a mixture of the 30- and 33-kD species with cyanogen bromide yielded three FGF-R1 peptides with NH_2-termini at residues P^{23}, P^{61}, and D^{129} (*7*). The latter COOH-terminal peptide had an apparent molecular size of 18 kD (Fig. 2D) and an amino acid composition that suggested that the peptide extended through F^{186} in loop III (*7*). The FGF-R1 heparin-binding domain was further localized by heparin-binding and protease protection analysis of a secreted recombinant fragment (Fig. 2E) that spanned the common FGF-R1 NH_2-terminus, loop I, the sequence between loop I and loop II, and the NH_2-terminal half of loop II of the three-loop FGF-R1α isoform (*3*, *8*). Interaction with heparin-agarose indicated that the resulting 56-kD fragment (*8*) contained a heparin-binding domain (Fig. 2E). Treatment of the immobilized fragment with lysine-specific endoproteinase Lys-C released a 23-kD peptide that reacted with monoclonal antibody M1C4 to an epitope within the 24 COOH-terminal residues of loop I (*3*) but not with monoclonal antibody M19B2, which reacts with an epitope shared by both FGF-R1α and FGF-R1β isoforms (*3*). Immunochemical analysis indicated that the parent undigested 56-kD fragment and a 27-kD fragment exhibiting the loop I M1C4, the acidic box, and the M19B2 epitopes remained bound to the heparin-agarose (Fig. 2E). The released 23-kD fragment probably spans the mature FGF-R1α NH_2-terminus through the first endo-Lys C-sensitive residue K^{50} (K^{139} in FGF-R1α), with 13 kD of mass attributable to amino acid content and 10 kD attributable to carbohydrate on the two N-linked sites of loop I (*2*, *3*). This indicated that neither loop I nor the acidic box contained a heparin-binding domain. The K^{50} residue is the partially protected tryptic cut site in FGF-R1β whose cleavage resulted in the 30-kD fragment with NH_2-terminus E^{51}TDNTKPN described above. Because the 27-kD fragment derived from the FGF-R1α construction that was retained on the heparin-agarose contained the complete α loop I sequence and the acidic box, we deduced that the FGF-R1 heparin-binding domain must lie downstream of K^{50} within the 4-kD difference in the COOH-terminal

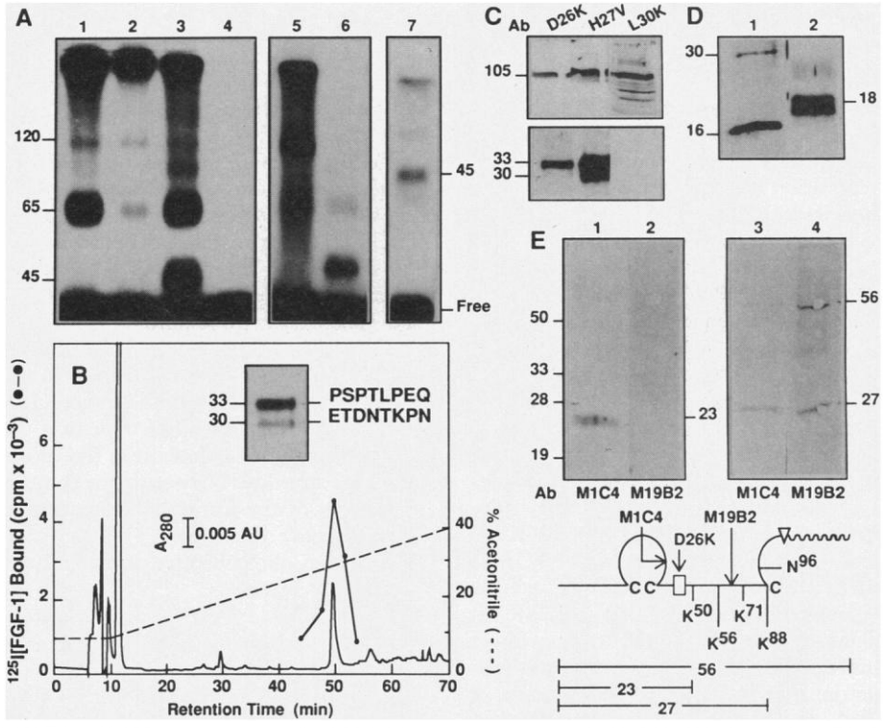

Fig. 2. Identification of the FGF-R1 heparin-binding domain. (**A**) Heparin-protection fragments of FGF-R1β. Infected Sf9 cell lysates (20 μl) containing FGF-R1β1 were mixed with PBS (lanes 1 and 2) or 1 ml of trypsin (0.5 μg/ml) in PBS (lanes 3 and 4) and incubated for 10 min at room temperature. Trypsin inhibitors were added, and the solution was then mixed with antibody A50 to FGF-R1 bound to protein A–agarose beads (*3*); ^{125}I-labeled FGF-1 was then bound and cross-linked to the immunocomplex. Assays in lanes 1 and 3 contained soluble heparin (2.5 mg/ml) during the trypsin treatment. In lanes 5 and 6, FGF-R1 was immobilized on heparin-agarose beads (10 μl), and the beads in lane 6 were subjected to the trypsin treatment before being used in binding assays. The labeled band at 120 kD is full-length 105-kD FGF-R1β1 with the 15-kD ligand. The band at 65 kD is ligand cross-linked to a 50-kD extracellular domain fragment resulting from proteolysis of the full-length FGF-R1β1 (*3*). Lane 7, ^{125}I-labeled FGF-1 bound to the purified heparin-protected fragment described in (B). (**B**) Purification of the heparin-protection fragments. Sf9 lysate containing FGF-R1β (25 to 50 μg) was applied to a heparin-agarose column (10 ml) in 0.1% Triton X-100 in PBS. Trypsin (200 μg) in 10 ml of 25 mM tris-HCl (pH 8.2) was applied to the column for 1 hour at 10°C. The column was washed with PBS containing 0.1% Triton X-100 and 0.2 mM phenylmethylsulfonylfluoride (PMSF) and then eluted with 2 M NaCl in the same buffer. The eluate was then applied to a 3-ml copper-chelating agarose column, and the column was washed with 0.5 mM imidazole in PBS and then eluted with PBS–5 mM imidazole. The eluate was then applied to a C4 reversed-phase column (0.46 × 25 cm) and eluted with the indicated gradient of acetonitrile. The active peak (50 μl of a 1-ml pool) was tested for ligand-binding activity (Fig. 2A, lane 7) and purity on silver-stained 12% SDS-PAGE (inset). For sequence analysis of the two bands, 500 μl were separated on SDS-PAGE, electrophoretically transferred to polyvinylidine difluoride (PVDF) membranes, and the indicated NH_2-terminal sequence of the excised bands determined by gas phase Edman degradation. (**C**) Immunochemical analysis of the 30- and 33-kD heparin-protection fragments. Sf9 cell lysate containing 100 ng of FGF-R1β1 (top) and 100 ng of purified heparin-protected fragments from the trypsin treatment (bottom) were analyzed after 12% SDS-PAGE and electrophoretic transfer to nitrocellulose paper (protein immunoblot) with the indicated rabbit polyclonal antibodies to peptide (*3*). (**D**) Lane 1, N-linked carbohydrate content of the 30-kD heparin-protection fragment. The purified 30-kD fragment was treated with 2 U of N-glycosidase F in 25 mM tris-HCl (pH 8.0) containing 0.5% CHAPS and then analyzed by protein immunoblot with antibody H27V. Lane 2, COOH-terminal cyanogen bromide peptide of the heparin-protection fragments (*7*). The peptide eluting at 27% acetonitrile from C18 reversed-phase HPLC with NH_2-terminus D^{129} was analyzed with antibody H27V. (**E**) Localization of the heparin-binding domain to the NH_2-terminus of loop II. Medium (18 ml) from Sf9 cells (5 × 10^6) infected with baculovirus bearing a partial cDNA coding for FGF-R1α (*8*) was mixed with heparin-agarose beads in PBS (1 ml). A 200-μl sample of the beads was mixed and incubated with 0.5 μg of endoproteinase Lys-C in 50 μl of 0.1 M tris-HCl (pH 8.5) and 1 mM $CaCl_2$ overnight at 37°C. The supernatant (lanes 1 and 2) and extract of the beads (lanes 3 and 4) were analyzed by 12% SDS-PAGE and immunoblot with the indicated monoclonal antibodies. M19B2 exhibits equal reactivity with FGF-R1α and FGF-R1β isoforms, but does not react with synthetic peptides D^{31} to K^{56} (D26K) or K^{71} to K^{88} (K18K) (*3*). Relevant domains and residues in the constructions are indicated in the diagram. The square is the acidic box, the triangle is the Eco RI site of FGF-R1, and the wavy line is the extension owing to in-frame ligation (*8*).

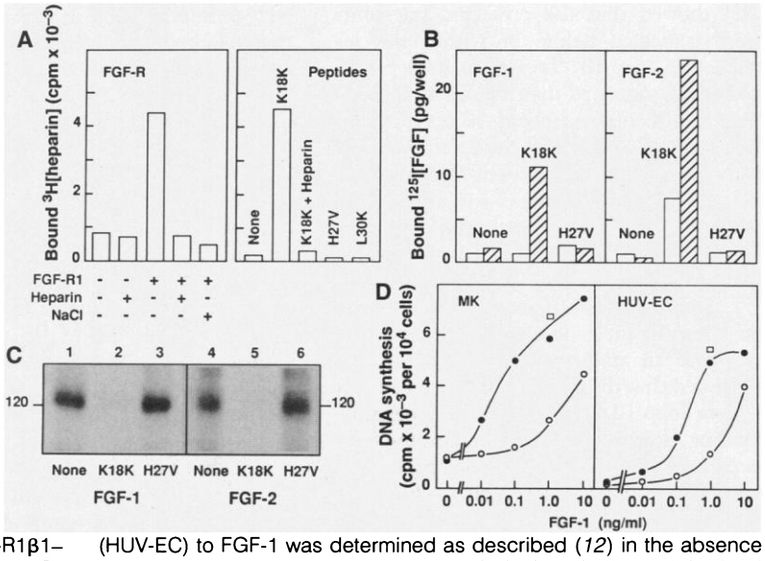

Fig. 3. Properties of the FGF-R1 heparin-binding sequence domain. (**A**) Heparin binding. (Left) Portions (50 μl) of infected (+) and uninfected (−) Sf9 cell lysates were mixed with antiserum A50 (10 μl) and protein A–beads (20 μl). The beads were washed with 0.6 M NaCl in PBS and then incubated with [^{3}H]heparin (2 μg/ml, 400 cpm/ng, 10 μl) for 1 hour at 10°C. Beads were incubated with unlabeled heparin or washed with 0.6 M NaCl before counting as indicated. (Right) Each of the indicated synthetic peptides (0.2 mg/ml) was immobilized in 96-well enzyme-linked immunosorbent assay plastic plates. Wells were incubated with 100 μl of [^{3}H]heparin for 1 hour at room temperature and washed three times with PBS. Bound heparin was extracted with 0.5 M NaOH and counted. Separate wells containing K18K were washed with unlabeled heparin before counting as indicated. (**B**) Ligand binding. Wells containing the indicated synthetic peptides were incubated stepwise with (hatched bars) and without (open bars) heparin (2 μg) and then with ^{125}I-labeled FGF-1 or FGF-2 (2 ng/ml). Bound radioactivity was extracted with NaOH and counted. (**C**) Antagonism of FGF receptor binding. The effect of the indicated synthetic peptides (200 μg/ml) on binding of ^{125}I-labeled FGF to immobilized complexes of heparin–FGF-R1β1–antibody–protein A–beads as shown in Fig. 1B (lane 6) was tested. (**D**) Antagonism of cell growth. The growth response (DNA synthesis) of mouse keratinocytes (MK) and human umbilical vein endothelial cells (HUV-EC) to FGF-1 was determined as described (*12*) in the absence (closed circles) and presence (open circles) of synthetic peptide K18K (20 μg/ml). Open squares indicate assays containing peptide H27V (200 μg/ml) instead of K18K. Data are the means of duplicate assays.

sequence of the released 23-kD and the retained 27-kD fragments. Moreover, the 4-kD COOH-terminus must end short of glycosylation site N^{96} within loop II, which otherwise would add 3 to 4 kD of additional size to the 27-kD retained peptide. The cluster of basic and hydrophobic residues K^{71}MEKKLHAVPAAKTVKFK^{88} (K18K) within the 4-kD sequence is characteristic of heparin-binding domains in heparin-binding proteins (9). The synthetic polypeptide K18K bound ^{3}H-labeled heparin (Fig. 3A), exhibited heparin-dependent FGF binding (Fig. 3B), and inhibited binding of FGF to the recombinant heparin–FGF-R1 complex (Fig. 3C) and to intact cells (Fig. 3D). Moreover, an antibody to the K18K sequence also inhibited binding of FGF to intact cells (Fig. 4A).

To test the function of the K18K sequence on binding of FGF to the intact FGF-R1 kinase, we substituted lysine residues in the sequence by mutagenesis of cDNA and examined the ligand-binding activity of expression products of the mutated cDNAs in transfected cells (Table 1). Substitution of lysine residues 71, 74, and 75 in the NH_2-terminus of K18K abrogated the binding of FGF, whereas substitution of COOH-terminal lysine residues 83 and 86 increased the apparent dissociation constant (K_d) (1764 pM) for FGF to over 13 times that of wild-type FGF-R1 (134 pM). Recombinant FGF-R1 in which the tandem lysine residues 74 and 75 were replaced in the NH_2-terminus of K18K bound 80% of the amount of FGF that bound to wild-type FGF-R1, whereas the substitution of Lys^{71} alone or with one of the two other NH_2-terminal lysines reduced binding to 10 to 30% that of wild type when FGF binding was compared in assays containing ^{125}I-FGF at the K_d of wild-type FGF-R1.

Finally, to determine whether the loss of ligand-binding activity as a result of mutation in the K18K heparin-binding domain correlated strictly with loss of heparin-binding activity of the receptor kinase, we directly tested the heparin-binding activity of heparin-binding domain mutant II (Table 1). The results suggest that ligand-binding activity closely correlates with the heparin-binding activity of the intact K18K sequence and that the K18K domain is the only heparin-binding domain in the receptor kinase from the NH_2-terminus through 18 residues of the intracellular juxtamembrane sequence (Fig. 4B).

Although our collective results do not establish that heparin or heparan sulfate glycosaminoglycan is part of the active FGF binding site of the FGF-R complex, they do indicate that the heparin-binding domain and associated glycosaminoglycan is essential for binding of FGF. Interaction of all three components may be obligatory for activation of signal transduction pathways linked to the FGF-R complex. Heparin, which probably mimics the native heparan sulfate components of the receptor duplex, appears to be a nonspecific requirement for binding of FGF homologs. Determination of whether specific heparan sulfate proteoglycans alter receptor complex specificity for different members of the FGF ligand family requires molecular characterization of the native heparan sulfate components of

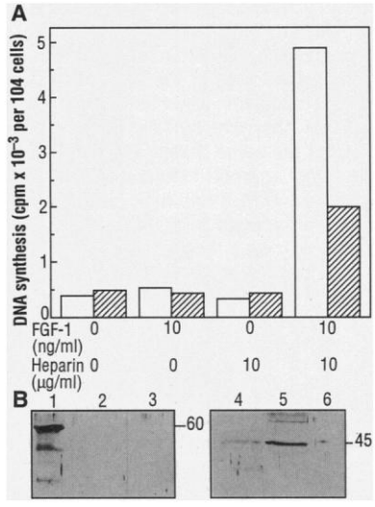

Fig. 4. (**A**) Inhibition of cell growth by antibody to K18K. An antibody to the synthetic K18K peptide was prepared in rabbits as described (*3*). The effect of 2 μl of immune serum (hatched bars) per 1-ml assay on DNA synthesis of human umbilical vein endothelial cells in the absence or presence of FGF-1 and heparin was compared to the effect of preimmune serum (open bars). (**B**) Loss of heparin-binding activity of K18K sequence mutant of FGF-R1. Heparin-binding site mutant II cDNA from Table 1 was expressed in COS-7 cells; the cells were extracted with 0.1% Triton X-100, and the extract analyzed by protein immunoblot with antibody A50 (lane 1). The same amount of extract was incubated overnight with heparin-agarose beads. Beads were eluted with 2 M NaCl and then 8 M urea and the eluates analyzed in lanes 2 and 3, respectively. Lanes 4 to 6 are positive controls for the analysis. The secreted product of a cDNA construction coding for FGF-R1 through I^{204}, which contained the wild-type K18K sequence from concentrated conditioned medium of transfected COS cells, was analyzed directly by immunoblot (lane 4). The same amount of product was incubated with heparin-agarose beads, which were then eluted with 2 M NaCl (lane 5) and then 8 M urea (lane 6).

Table 1. Effect of lysine substitutions in the heparin-binding domain of FGF-R1 on ligand binding. Mutagenesis mediated by polymerase chain reaction (*14*) was used to generate point mutations in cDNA, resulting in the substitutions of the underlined amino acids (*5*) that are residues in the same spatial positions in the sequence of Ig-like loop I of FGF-R1α (*3*). FGF-R1β1 template was used with 5′-primer P1a, which is 67 base pairs (bp) upstream of the translational initiation site, 3′-primer P1b2, which begins one codon downstream of the codon for the COOH-terminal cysteine of loop II, and complementary pairs of primers containing the mutations to generate a 500-bp cDNA (*2, 14*). After treatment with Klenow enzyme and Bst XI, the purified cDNAs were ligated at the Bst XI site with a 600-bp FGF-R1β cDNA extending through the sequence coding for 18 residues of the intracellular juxtamembrane (*2, 14*). Complementary DNAs (1 kilobase pair) were cloned into pBluescript SK vector at Eco RI and Eco RV sites, verified by sequence, and then cloned into mammalian expression vector P91023B (*2, 3, 14*). Transfected COS-7 cells were split into 24-well plates (8×10^4 cells per well) for replicate ligand- and antibody (H27V and A50)-binding assays (*14*). Binding assays contained 120 pM FGF-1 or FGF-2 labeled to a specific activity of 3×10^5 cpm per nanogram. The amount of ligand and antibody bound to untransfected cells was subtracted from that bound to transfected cells. Ligand binding among cells transfected with different constructions was normalized to amount of cell surface antigen expressed by the quotient of the amount of specifically bound ligand divided by the amount of specifically bound antibody. Ligand binding was then expressed as a percentage of that in cells transfected with wild-type FGF-R1β cDNA (I). Scatchard analysis of FGF-1–binding to cells transfected with wild-type (I) and mutants II, III, and IV constructions were performed, and the indicated apparent K_d calculated as described (*2, 12*). ND, not determined.

	Sequence						Binding (%)		K_d (pM)
							FGF-1	FGF-2	
I	K^{71}	MEK^{74}	K^{75}	$LHAVPAAK^{83}$	TVK^{86}	FK^{88}	100	100	134
II	P	MEV	E	LHAVPAAK	TVK	FK	<10	<10	—
III	K	MEK	K	LHAVPAAD	TVQ	FK	40	55	1764
IV	K	MEV	E	LHAVPAAK	TVK	FK	80	80	310
V	P	MEK	E	LHAVPAAK	TVK	FK	20	10	ND
VI	P	MEV	K	LHAVPAAK	TVK	FK	20	20	ND
VII	P	MEK	K	LHAVPAAK	TVK	FK	30	25	ND

the FGF-R complex from different cells and tissues. Native heparan sulfate proteoglycans that react with FGF have been reported (*10*). One in particular appears to copurify with the tyrosine kinase glycoprotein component of the FGF-R complex (*11*). Isolation and characterization of the native tissue- and cell-specific heparan sulfate proteoglycan cofactors of the FGF-R complex with the recombinant glycoprotein tyrosine kinase component as an affinity probe should clarify this question.

REFERENCES AND NOTES

1. M. Kan and E. Shi, *In Vitro Cell. Dev. Biol.* **26**, 1151 (1990); A. Yayon, M. Klagsbrun, J. D. Esko, P. Leder, D. M. Ornitz, *Cell* **64**, 841 (1991); A. C. Rapraeger, A. Krukfa, B. B. Olwin, *Science* **252**, 1705 (1991).
2. J. Hou *et al.*, *Science* **251**, 665 (1991).
3. J. Xu *et al.*, *J. Biol. Chem.* **267**, 17792 (1992).
4. Heparin-like cell-surface binding sites are molecules that bind ^{125}I-labeled FGF but that cannot be covalently cross-linked to it (*12*). Ligand bound to the sites can be extracted with 2 M NaCl or heparin after termination of binding assays (*12*). HepG2 cells display about 1×10^6 such sites for FGF-1 with apparent K_d of 600 pM. Mild treatment with trypsin reduced heparin-extractable FGF-1 binding by 88%. Heparin-like FGF-binding molecules were released from 1×10^7 HepG2 cells by treatment with 4 ml of trypsin (25 μg/ml) for 10 min at 4°C. After the addition of trypsin inhibitors, the released material was dialyzed against water, freeze-dried, and reconstituted in 250 μl of phosphate-buffered saline.
5. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. Amino acid residues in FGF-R1 are numbered from the first methionine in the two Ig-loop FGF-R1β isoform (*3*). Loops I, II, and III refer to the three Ig-like loops of the FGF-R1α isoform, which is distinguished from FGF-R1β by the presence of the 89-residue NH_2-terminal loop I inserted between residues Q^{30} and D^{31} of FGF-R1β (*3*).
6. Polyclonal rabbit antiserum L30K was prepared to a 29-residue synthetic peptide corresponding to the FGF-R1β sequence L^{222} to Y^{250} (*3*). Analysis of enzymatic cleavage fragments of the peptide revealed that the antiserum reacts only with the COOH-terminal residues of the peptide corresponding to FGF-R1β residues E^{233} to Y^{250} (*3*).
7. A mixture of the purified 30- to 33-kD heparin-protected fragments was cleaved by treatment with cyanogen bromide in 50% formic acid, and the resulting products were analyzed on a C18 reversed-phase column (0.46 × 25 cm) with a gradient of 10 to 70% acetonitrile in 0.1% trifluoroacetic acid. Three peaks, which eluted at 39, 43, and 52% acetonitrile, resulted from each of three independent experiments and exhibited NH_2-terminal sequences P^{23}(S)PTLPEQ, P^{61}VAPY-(S)TSPEKM^{72}EKKLH, and D^{129}SVVPSDKG(N)YT-(C)IVE, respectively. Residues in parentheses were unclear in the sequence analysis. The Met^{72} residue is apparently resistant to cleavage. Two independent amino acid composition analyses of the latter COOH-terminal peptide of the fragments indicated a mean Phe content of 1.9%, as compared with an expected 1.7% for the complete cyanogen bromide peptide that would extend through $F^{186}M^{187}$. There is no other Phe residue in FGF-R1β preceding the E^{233} to Y^{250} epitope.
8. A partial FGF-R1α cDNA coding for the translational initiation site, loop I, the sequence between loop I and loop II containing the acidic box and half of loop II through F^{108} (which ends at the single Eco RI site of FGF-R1β) was cloned into the Eco RI sites of baculovirus transfer vector pVL1392, and recombinant baculovirus was prepared as described (*3*). A 56-kD secreted glycoprotein product resulted from infected Sf9 cells because of in-frame ligation with the baculoviral transfer vector.
9. A. D. Cardin and H. J. R. Weintraub, *Arteriosclerosis* **9**, 21 (1989).
10. S. Saunders, M. Jalkanen, S. O'Farrell, M. Bernfield, *J. Cell Biol.* **108**, 1547 (1989); P. Marynen, J. Zhang, J. J. Cassiman, H. Van den Berghe, G. David, *J. Biol. Chem.* **264**, 7017 (1989); M. C. Kiefer, J. C. Stephans, K. Crawford, K. Okino, P. J. Barr, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 6985 (1990).
11. K. Sakaguchi, M. Yanagishita, Y. Takeuchi, G. D. Aurbach, *J. Biol. Chem.* **266**, 7270 (1991).
12. M. Kan *et al.*, *ibid.* **263**, 11306 (1988); M. Kan, E. Shi, W. L. McKeehan, *Methods Enzymol.* **198**, 158 (1991).
13. Portions containing receptor (5 to 10 ng) from detergent extracts of Sf9 insect cells infected with recombinant baculovirus coding for FGF-R1β1 (*flg*) (20 μl) (*3*) were mixed with protein A–agarose (10 μl) and rabbit antiserum A50 (5 μl) to a bacterial-derived recombinant FGF-R1α extracellular domain (*3*). Where indicated, the beads were incubated sequentially with 0.6 M NaCl, heparin or heparan sulfate proteoglycan (HS) (250 μg/ml), and again with 0.5 M NaCl before binding of ^{125}I-labeled FGF (2 ng/ml). Beads were collected between each incubation by centrifugation and washed with PBS. A 100-fold excess of unlabeled FGF-1 or FGF-2 was added to binding assays as indicated. After termination of binding assays, beads were washed with PBS, then with PBS containing heparin (250 μg/ml), and then again with PBS before covalent cross-linking and analysis by 7.5% SDS-PAGE and autoradiography.
14. J. Hou *et al.*, *J. Biol. Chem.* **267**, 17804 (1992).

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