FGF2-Heparin Co-crystal-assisted Design of Mutants FGF1 and FGF7 with Predictable Heparin Affinities*

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The co-crystal structures of FGF2 and heparin-derived tetra- and hexasaccharides demonstrated the existence of high and low affinity contact residues that are likely to be involved in heparin binding (Faham, S., Hileman, R. E., Fromm, J. R., Linhardt, R. J., and Rees, D. C. (1996) Science 271, 1116–1120). To study the role of these putative contact residues, we chose three fibroblast growth factor family members with distinct heparin affinities for comparative mutagenesis studies. Only one amino acid significantly differed between FGF1 and FGF2 and was mutated, FGF1-31K. FGF7, also called keratinocyte growth factor, was mutated to mimic either FGF1 or FGF2 at two of the putative high contact points termed FGF7-1 and FGF7-2, respectively. FGF2 has higher apparent heparin affinity than FGF1 or FGF7, and FGF1 has higher heparin affinity than FGF7. All three mutants showed an increase in apparent heparin affinity compared with wild types. FGF7-1 has a lower apparent heparin affinity than FGF7-2, analogous to wild type FGF1 and FGF2. The FGF1-31K mutant showed no change in mitogenic activity, whereas the FGF7 mutants exhibited a decrease in activity. These results indicate that the co-crystal structure of the FGF2-heparin complexes can be used to design a rational approach to the generation of mutants with defined affinities for heparin or heparan sulfate proteoglycans.

The fibroblast growth factor family (FGF) present consists of at least 10 structurally related proteins (1–4). FGF1 and FGF2, also commonly referred to as acidic and basic FGF, respectively, are the prototypes of the family and are the most extensively characterized. The FGF family members share a wide variety of overlapping biological activities and receptor binding properties. They are also capable of binding to heparin and heparan sulfate proteoglycans (HSPGs). It has been demonstrated that heparin can potentiate the mitogenic activity of FGF1 (5, 6) and protect it from acid, heat, and proteolytic inactivation (7–9). Heparin also increases the apparent affinity of FGF1 and FGF2 for the high affinity FGF receptors (10–12). It has been suggested that 2–10 saccharide units are capable of binding to FGF2, but 8–12 saccharide units are required for receptor dimerization and subsequent activation (13, 14). Heparin and HSPGs also have been shown to bind and internalize FGF2 (15, 16).

Heparin and HSPGs are negatively charged polymers (13) and are thought to interact with protein molecules primarily via electrostatic interactions. However other factors such as amino acid composition, positioning, and conformation are likely to contribute to the specificity of heparin-protein interactions. Margalit et al. (17) employed computer modeling and sequences of known heparin-binding proteins to determine the spatial distribution that may affect heparin binding. From their analysis of full-length human FGF1, basic amino acids corresponding to positions 126 and 133 were determined to be key residues for heparin binding. However, residue 133 is not conserved in different species of FGF1. In bovine and chicken, this residue is a leucine, and these proteins exhibit heparin affinities that are similar to that of human FGF1 (18).

It also has been suggested that the heparin binding domain on proteins can be defined through linear consensus sequences (19). The proposed motifs of XBBXXB or XBBBXXBX, where B designates a basic residue and X a hydrophobic amino acid, corresponds well with three regions in the primary sequence of FGF1. These regions include residues 22–27, 113–120, and 124–131. We recently examined the role of basic residues in these regions in FGF1-heparin interactions by site-directed mutagenesis of the basic amino acids to glycine residues (20). Mutations of basic residues between 22–27 generated no effect, but mutations within residues 113–120 did result in a slight decrease in apparent heparin affinity. The most dramatic decrease within these regions was seen at position 127. However when lysine 132, which is located just outside of the putative consensus sequence, is mutated, the apparent heparin affinity of FGF1 is reduced significantly (6).

The importance of residues 127 and 132 in heparin binding is supported by the co-crystal structure of FGF2 and heparin-derived tetra- and hexasaccharides. Faham et al. (21) identified high and low affinity contact residues on FGF2 that are involved in heparin binding. A protein sequence alignment between these contact residues of FGF2 and the FGF family members identified residues in FGF1 corresponding to amino acids 127 and 132 as putative high affinity contact sites. In this alignment, the two residues predicted by computer modeling to be involved in heparin binding did not correlate with either high or low affinity contact points. Consistent with the co-crystal structure, when we mutated these residues identified by molecular modeling, there was little effect on heparin binding (20).

From this sequence alignment, there were no residues that
were entirely conserved throughout the FGF family, yet all members are capable of binding heparin. We chose three members from the FGF family, each with a distinct heparin affinity, to study the role of these contact residues in heparin binding. FGF2 has higher apparent heparin affinity than FGF1 and FGF7, which is also known as keratinocyte growth factor. FGF7 has lower apparent heparin affinity than FGF1. We examined the effect on heparin affinity when these putative contact points are mutated to resemble other FGF family members. The results are consistent with the co-crystal structure of heparin-derived tetra- and hexasaccharides and FGF2 and suggest that a rational approach to generating mutants with designed heparin and heparan sulfate proteoglycan affinities is possible.

**EXPERIMENTAL PROCEDURES**

**Materials**—Heparin-Sepharose and low molecular weight protein markers were purchased from Amersham Pharmacia Biotech. All reagents for polyacrylamide gel electrophoresis and the Mighty Small Electrophoresis and transfer apparatus were from Hoefer Scientific Instruments (San Francisco, CA). The E. coli EcoRI cloning vector (5.8 kb) were purchased from Bio-Rad. Reagents for reverse phase HPLC were from Applied Biosystems Inc. (Foster City, CA). Amino acid analysis reagents were purchased from Waters Associates (Medford, MA). Chloramine T and sodium metabisulfate were obtained from Sigma. [3H]Thymidine and Na[1-14C] were purchased from Amersham. Eagle’s minimal essential medium, Dulbecco’s modified Eagle’s medium, calf serum, penicillin-streptomycin, t-glutamine, and Ham’s F-12 media were purchased from Biofluids (Rockville, MD). Dyalkylated fetal bovine serum and selenium were from Sigma. Transferin was from Intergen (Purchase, NY), and human epidermal growth factor was obtained from UBI (Lake Placid, NY). Heparin (6.15 μg/unit) was purchased from The Upjohn Co. Other chemicals were reagent grade.

**Construction of Wild Type and Mutant FGF1 Prokaryotic Expression Plasmids**—Full-length FGF1 cDNA was a gift from Dr. Mike Jaye (Rhone-Poulenc Rorer Central Research). The pMJ17 plasmid containing β-FGF1 was digested with EcoRI and HindIII to generate a 1000-bp pair fragment with the EcoRI site at the 5′ end. This fragment was subjected to a 1% agarose gel electrophoresis, excised from the gel, purified by Wizard DNA Clean-up System (Promega, Madison, WI), and subcloned into the EcoRI and HindIII sites of pBluescript SK+ (Strategene, La Jolla, CA). After ligation, the pBluescript SK+ was transfected into E. coli DH1 and expressed by isopropyl β-D-thiogalactopyranoside. The FGF1-31K mutant was generated using the wild type plasmid with the NdeI restriction site and the mutagenic primer 5′-CCT CTA CCT TCA GCC ATA TGT CAG CAG C-3′. The FGF1-31K mutant was digested with EcoRI and HindIII digestion and agarose gel electrophoresis. Site-directed mutants were generated using the Mutagen-Phase Geneid Mid In Vitro Mutagenesis kit version 2 (Bio-Rad) as described previously (25). NcoI and BamHI restriction site were introduced into the 5′ and 3′ of the DNA fragment using the following primers: (sense) 5′-CGA GGG ATT CCT AAA AGA GG-3′; and (antisense) 5′-GGG TTC TTT ATA TAC CGG ATC CAA TTA ATG TAT TGC-3′, respectively. Samples were subjected to 35 cycles of amplification using a Perkin-Elmer 9600 thermocycler. Each cycle included denaturation at 94°C for 40 s, annealing at 58°C for 40 s, and primer extension at 72°C for 40 s. The resulting fragment was subjected to agarose gel electrophoresis, excised from the gel, and electro-eluted as described previously (26). The FGF7 fragment was cloned using the pCR-Script SK+ cloning kit (Strategene). The FGF7 mutants were generated as described above for FGF1 using the following primers: 5′-GCC TTA AAT CCT AAA AGA GG-3′; and 5′-GCC TTA AAT CGA AAG GGG ATT CCT AAA AGA GG-3′. The FGF7s were subcloned into the NcoI and BamHI sites of the pET15b (Novagen, Madison, WI) expression vector as described previously (24).

**RESULTS**

**Generation of Wild Type and Mutant FGF1 and FGF7**—Overnight cultures of E. coli BL21(DE3) pLysS cells containing recombinant plasmid were diluted into 1-liter cultures of Luria broth containing 50 μg/ml ampicillin and 34 μg/ml chloramphenicol and allowed to grow to an absorbance of 0.3 at 600 nm. Isopropylthiol β-galactosidase was then added to a final concentration of 1 mm, and the cultures were incubated at 37°C for an additional 2 h. Cell pellets were collected by centrifugation and stored at −80°C until further use. Cell pellets from 1-liter cultures were resuspended in 50 ml of 50 mM Tris and 10 mM EDTA, pH 7.4. The cells were incubated on ice for 30 min, then sonicated at maximum intensity for 30 s using the large probe of a Heat System 380 sonicator. Lysates were clarified by centrifugation at 6,000 × g for 20 min at 4°C. The supernatants were diluted to 100 ml with 50 mM sodium phosphate, pH 7.4 (buffer A), and applied to a 5-ml Bio-Rad heparin cartridge using a Waters Associates HPLC system. The proteins were eluted with a linear gradient of buffer A and buffer B containing 2.0 mM NiCl. Flow was 1.5 ml/min, and 1 ml fractions were collected.

**Western Blot Analysis**—Aliquots of fractions eluted from the heparin cartridge were subjected to SDS-polyacrylamide gel electrophoresis as described by Laemmli (26). A 15% acrylamide and 0.4% N,N′-methylenebisacrylamide solution was polymerized in a Hoefer Mini-gel apparatus, and electrophoresis was carried out at 200 V. Proteins were transferred from the gel to nitrocellulose in 25 mM Tris, 0.2 mM glycine, 20% methanol, and 0.02% SDS for 30 min at 50 V, and an additional 60 min at 100 V. FGF1- and FGF7-containing fractions were identified by Western blotting with rabbit polyclonal FGF1-specific antibody or FGF7–terminal-specific antibody, respectively, and 125I-protein A as described previously (27). The FGF1 and FGF2 antibody were provided by Dr. Dan Rifkin (Department of Cell Biology, New York University Medical Center). FGF7 and FGF7 antibody were kindly provided by Drs. Don Bottaro and Jeff Rubin (Laboratory of Cellular and Molecular Biology, National Cancer Institute).

**Mitogenic Assays**—Balb MK cells were grown in 96-well plates at 37°C to ~80% confluency in low calcium Eagle’s minimum essential medium containing 10% dialyzed fetal bovine serum and 5 ng/ml epidermal growth factor. The cells were serum-starved in a 1:1 mix of Ham’s F-12 and Eagle’s minimum essential medium containing 5 μg/ml transferrin and 30 ng/ml selenium for 72 h. Selenium and transferrin are trace elements found in serum whose presence is required for cells to respond to the addition of growth factors after being starved (28). Serum media was changed before the addition of growth factors. After 18 h, the cells were pulsed with [3H]thymidine (1 μCi/ml). The cells were harvested 4 h later using a Filter Mate Cell Harvester (Packard Instrument Co., Inc.) onto a glass fiber filter (Packard Instrument Co., Inc.), and [3H]thymidine incorporation into DNA was measured using a Matrix 9600 Direct beta counter for 3 min.

**RESULTS**

**Generation of Wild Type and Mutant FGF1 and FGF7**—Specific residues in FGF1 have been identified previously to have a substantial impact on its apparent heparin affinity (20, 29). Recently, the co-crystallization between FGF2 and heparin-derived tetra- and hexasaccharides identified residues involved in high and low affinity binding to heparin (21). These high and low affinity residues, when compared with additional FGF members in a protein sequence alignment as shown in Table 1, support other work identifying putative heparin binding residues in FGF1 (27, 29). FGF2 has higher apparent heparin affinity than FGF1, and from the FGF1-31K mutation, FGF2 has a substantially different apparent heparin affinity than either FGF1 or FGF2, varied the most in the high affinity contact points between FGF1 and FGF2. Mutants of FGF7 were generated to mimic either FGF1 or FGF2
FGF Mutants with Predictable Heparin Binding Affinities

TABLE I

High and low affinity contact residues of FGF2 and corresponding residues of other FGF family members

| FGF family member | Residue number |
|-------------------|---------------|
|                   | 31  | 32  | 109 | 127 | 132 | 141 | 142 |
| FGF-1             | Ser  | Asn | Asn | Lys | Lys | Gln | Lys |
| FGF-2             | Lys  | Asn | Asn | Arg | Lys | Gln | Lys |
| FGF-7             | Arg  | Thr | Asn | Gln | Val | Gln | Lys |
| Affinity          | Low  | High| Low | High| High| High| Low |

High affinity contact residues are defined by the co-crystallization of heparin-derived tetrasaccharide interactions with FGF2. Low affinity sites are defined as the additional contact sites when using heparin-derived hexasaccharides. FGF1 is the reference sequence where 1 is the first residue after the initiator methionine. FGF family sequence alignment was done using the Wisconsin Sequence Analysis package.

by altering the high affinity positions glutamine 127 to lysine or arginine, respectively, and valine 132 to lysine, which is common to both FGF1 and FGF2. These mutants were termed FGF7-1 and FGF7-2, respectively.

The FGF1 DNA coding sequence was originally cloned into the pUC9 vector with EcoRI (30). The insert was digested with EcoRI and HindIII, which removed a large portion of the 3′-untranslated region and was subcloned into pBluescript SK+. Single-stranded DNA was generated (22), and an NdeI site was introduced as the initiator methionine for subcloning into the pET3c expression vector (Fig. 1). Mutant FGF1-31K was generated from the wild type FGF1 sequence containing the NdeI restriction site in pBluescript SK+ and subcloned into pET3c as shown in Fig. 2 (22). The FGF1 proteins were expressed in BL21(DES) pLysS cells. FGF7 is a secreted protein with a classical signal sequence (31). To obtain the mature form of the secreted protein, the 5′ primer was designed with an NcoI site as the initiator methionine as described previously in Ron et al. (32). The FGF7 DNA sequence was obtained from human fibroblast cDNA using the polymerase chain reaction method (25). The polymerase chain reaction product was cloned into the pCR-Script SK+ vector. The NcoI site and a BamHI site at the 3′-untranslated region were used to subclone FGF7 into pET15b (Fig. 1). FGF7-1 and FGF7-2 mutants were generated to have the putative heparin contact residues corresponding to FGF1 or FGF2, respectively (Fig. 2). These proteins were expressed in BL21(DES) pLysS cells.

Altered Heparin Affinity of FGF1 and FGF7 Mutants—All members of the FGF family are capable of binding to heparin and heparan sulfate proteoglycans, but their relative affinities vary. From a protein sequence alignment, a comparison shows that none of the putative contact points are conserved throughout the family (21). This indicates that the differences in heparin affinities between family members may result from differences in the individual contributions of these amino acids to heparin binding. The heparin affinities of wild type and mutant FGF proteins were assayed by the concentration of NaCl required to elute the proteins from immobilized heparin.

There is only one amino acid of these putative contact residues, amino acid Lys-31, that differs substantially between FGF1 and FGF2, yet FGF2 has significantly higher apparent affinity for heparin. Recombinant wild type FGF2 eluted from immobilized heparin at 1.23 M NaCl (Fig. 3). The FGF1 site-directed mutant was generated to contain a lysine at position 31, as in FGF2, rather than a serine residue. To avoid pre-selecting high or low affinity heparin binding FGFs, clarified E. coli lysates were applied to immobilized heparin. Western blot analysis performed on fractions of FGF1 collected from immobilized heparin showed that wild type FGF1 eluted in the range of 0.92 to 1.10 M NaCl. The FGF1-31K mutant showed an increase in apparent heparin affinity with an elution range between 0.98 to 1.22 M NaCl (Fig. 3).

FGF7 exhibits two putative high affinity contact residues dramatically differing from the rest of the FGF family members. These were at positions 127 and 132. The FGF7 mutants were designed to mimic the putative heparin contact residues in FGF1 and FGF2. Western blot analysis of fractions collected from immobilized heparin showed that wild type FGF7 was eluted from the column in the range of 0.64–0.84 M NaCl (Fig. 4). The FGF7-1 and FGF7-2 mutants exhibited increased apparent heparin affinity. FGF7-1 eluted in the range of 0.80–0.98 M NaCl and FGF7-2, between 0.92 and 1.10 M NaCl. Analogous to wild type FGF1 and FGF2 apparent heparin affinities, the FGF7-1 mutant had lower apparent heparin affinity than the FGF7-2 mutant (Fig. 4).

Mitogenic Activities of FGF1 and FGF7 Mutants—It has been shown previously that diminishing heparin affinity can alter mitogenic activity. An FGF1 mutant, where a glutamic acid is substituted for a lysine at residue 132, has reduced heparin affinity but is still able to bind to receptor and stimulate the immediate early genes c-fos and c-jun, similar to wild type. However [3H]thymidine incorporation is greatly reduced, suggesting that relatively high affinity heparin binding is re-
quired for completion of the mitogenic signal of FGF1 (6). To determine whether these mutants also have altered capacity to stimulate mitogenesis, DNA synthesis assays were performed on Balb MK cells. The FGF1-31K mutant showed no altered capacity to stimulate DNA synthesis compared with wild type. For both, the half maximum was at 1 ng/ml, and maximum stimulation occurred at 10 ng/ml (Fig. 5). There is a decrease in [3H]thymidine incorporation at high concentrations of both wild type and mutant FGF1 that may be due to high FGF receptor occupancy that does not allow for FGF receptor activation. It is also possible that FGF1 at this concentration is acting in another function that is not detected by [3H]thymidine incorporation.

FGF7 activity is restricted to cells expressing a splice variant of FGFR-2 (33, 34) and has been shown to be inhibited by the presence of heparin (32, 35). The mitogenic assays were performed in the absence of heparin to allow for maximum activity. The results show that both FGF7-1 and FGF7-2 mutants are slightly less active than wild type. When compared with each other, FGF7-1 is slightly more active than FGF7-2.

FIG. 2. FGF1 and FGF7 wild type and mutant sequences. The full-length amino acid sequence of human FGF1 and the amino acid sequence of the secreted form of FGF7 are shown. The amino acids that have been mutated by site-directed mutagenesis are indicated. The boxed residues are those previously mutated by site-directed mutagenesis (20). Amino acid residues are identified using the single letter code and numbered as referenced by wild type FGF1.

FIG. 3. Heparin binding properties of wild type human FGF1 and FGF2 and the site-directed point mutant of human FGF1. Recombinant wild type and mutant FGF1s were generated as described under “Experimental Procedures.” Lysates of E. coli producing wild type and mutant FGF were subjected to heparin affinity-based chromatography using a linear gradient of 0–2.0 M NaCl. Eluted fractions were collected and subjected to Western blot analysis. Autoradiograms of selected fractions of wild type and mutant FGF1 are shown along with the range of NaCl concentrations required for elution.

FIG. 4. Heparin binding properties of wild type and site-directed point mutants of FGF7. Recombinant wild type and mutant FGF7 were generated as described in “Experimental Procedures.” Lysates of E. coli producing either wild type or mutant FGF7 were subject to heparin affinity-based chromatography. Wild type and mutants were eluted using a linear gradient of 0–2.0 M NaCl. 1-min fractions were collected and subjected to Western blot analysis for FGF7 immunoreactivity. Autoradiograms of selected fractions of wild type or mutant FGF7 are shown.
DISCUSSION

Previous work identifying regions involved in heparin binding of FGF1 demonstrated the importance of residue 132 (6, 29). Site-directed mutagenesis of this residue from a lysine to a glutamic acid showed this mutant to have reduced affinity toward immobilized heparin and to be a poor mitogen (6). However it was able to bind its high affinity receptors and induce the immediate early genes c-fos, c-jun similar to wild type FGF1 (27). This demonstrates a dissociation between activation of immediate early events and heparin binding of FGF1.

The heparin binding domain on FGF1 has been suggested to be defined by a linear consensus sequence of basic and hydrophobic amino acids by Cardin and Weintraub (19). We previously examined the role of these basic residues identified by the consensus sequences for their impact on heparin binding. There are three regions in FGF1 that fit well with the XBBXBX or XBBBXXBX motifs and they are residues 22–27, 113–120, and 124–131. Residue 132 lies immediately outside any of these consensus motifs. Using site-directed mutagenesis, the boxed basic residues in Fig. 2 were changed to glycine. Mutations in region 22–27 and 113–120 had little to no impact on affinity for immobilized heparin. Residue 127 within region 3 had a significant effect on apparent heparin affinity, although the adjacent residue, mutant 126 had a modest effect (20).

Other site-directed mutants have been generated where basic residues replaced nonbasic residues between amino acids 124 and 131. None of these mutants had increased heparin affinity but retained affinities similar to wild type.2 These results indicate that these residues are not involved in heparin binding and that an increase in the number of basic residues on the protein surface is not sufficient in affecting apparent heparin affinity.

The co-crystal structure between FGF2 and heparin-derived tetra- and hexasaccharides identified residues that are involved in high and low affinity binding. These amino acids do not form linear sequences but are interspersed throughout the protein. When these basic amino acids are aligned with other FGF family members, the occupancy of these positions with basic residues seems to correlate with increased heparin affinity, although none had been tested (21). Previous work identified positions 127 and 132 as having significant impact on FGF1 heparin affinity (20, 29) and, interestingly, the protein sequence alignment of high and low affinity contact sites identified these two positions as two high affinity positions. FGF7 varied the most at these two positions.

To determine whether these contact points are involved in heparin affinity in other FGF family members and whether heparin affinity can be altered in a predictable way based solely on the occupancy of these contact residues, we chose three FGF family members to study, each with a distinct heparin affinity. The putative contact residues for FGF1, FGF2, and FGF7, shown in Table I, suggest that basic residues at specific positions are involved in high affinity binding to heparin. FGF2 has higher apparent heparin affinity than FGF1. The only substantial difference between FGF1 and FGF2 is amino acid 31. Using site-directed mutagenesis, we modified this putative low affinity site on FGF1 to mimic FGF2, serine 31 to lysine. The results show the FGF1-31K mutant has an increased apparent heparin affinity compared with wild type. The other mutants showed similar results. FGF7 has the lowest apparent heparin affinity between FGF1 and FGF2. Two high affinity mutations, amino acids 127 and 132, were constructed to correspond to the FGF1 and FGF2 contact points. Both FGF7-1 and FGF7-2 mutants exhibited increased apparent affinity for immobilized heparin, and the relative affinities were analogous to wild type.

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2 P. Wong and W. H. Burgess, unpublished results.
FGF1 and FGF2 affinities. The results indicate that amino acids 127 and 132 in FGF2 and FGF7 are involved in heparin binding. The difference in increased apparent heparin affinity between FGF7-1 and FGF7-2 may be due to the type of basic residue substitution. Fromm et al. (37) demonstrated that a seven-residue arginine peptide had higher affinity than a seven-residue lysine peptide for immobilized heparin. These results indicate that the properties of the amino acid involved in heparin binding also is important in defining heparin affinity. FG1 and FGF2 consists of 12 antiparallel β-sheets (36); however, the crystal structure of other FGF family members have not been solved. These results indicate that there may be a generality to the co-crystal structure of FGF2 and heparin-deprived saccharides that extends to other members of the family.

The biological activities of these mutants, as measured by stimulation of DNA synthesis, show that they can be altered through their heparin binding properties. As previously shown, a FGF1–132E mutant that had decreased heparin affinity was able to stimulate tyrosine phosphorylation and activate immediate early genes. However, this mutant was unable to complete the mitogenic signal, indicating a role for heparin in FGF1 activity. Exogenously added heparin inhibits the mitogenic response of FGF7 (32), suggesting that the diminished mitogenic activity of these FGF7 mutants may be a result of increased affinity for cell surface HSPGs. These two contrasting effects of heparin in the mitogenic activity of FGF may be another mechanism by which FGF activity is regulated. These results also demonstrate that it is possible that FGF mutants can be generated and targeted to specific areas as agonists or antagonists by modifying their heparin binding properties.

Together, these results and the results of previous studies indicate that for the FGF family, a heparin binding domain does not consist of a linear sequence of amino acids and cannot be defined by a consensus sequence. The co-crystal structures of FGF2 and tetra- and hexasaccharides identified key residues that generate high and low affinity contact points. In the linear protein sequence, these residues are interspersed, and once folded to form a tertiary structure, generate a high affinity heparin-binding protein. Previously, we and others identified a full-length protein is derived by the amino acids on the surface of a folded protein.

The results support the co-crystal structure of heparin derived tetra- and hexasaccharides with FGF2 as a structure consistent with solution studies. The data also demonstrate that the co-crystal structure of the FGF2-heparin complexes can be used to design a rational approach to generate mutants of other FGF family members with defined heparin and HSPGs affinities.

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