Engineering of Fibroblast Growth Factor: Alteration of Receptor Binding Specificity†

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ABSTRACT: A five-residue loop structure in basic fibroblast growth factor (FGF-2) which extends from amino acid residue 118 to residue 122 was replaced, by cassette mutagenesis, with the corresponding seven-residue loop structure from the structural homologue acidic fibroblast growth factor (FGF-1) or the corresponding five-residue loop from interleukin-1β to give FGF-2LA and FGF-2LI, respectively. The mutants were expressed in *Escherichia coli* and purified to homogeneity, and their heparin and receptor binding and biological properties were examined. The ability of FGF-2LA to induce endothelial cell proliferation was the same as that of FGF-2. Affinities of the mutants to heparin and to cells that express FGF receptor-1 (FGFR-1) were identical to those of the wild-type protein. The role of the loop structure in FGF-1 and FGF-2 was elucidated by using soluble FGF receptor systems, which display distinct ligand binding specificities. Thus, FGF-2LA bound, with the same affinity as FGF-1 and FGF-2, to FGFR-1 and FGFR-2, whereas only FGF-1 and the FGF-1 loop-containing mutant, FGF-2LA, bound to the keratinocyte growth factor receptor. A change in receptor binding specificity was not observed with the FGF-2LI engineered mutant. That the binding specificity of FGF-2 was dramatically altered by transfer of a loop structure from FGF-1 to resemble the binding profile of the donor protein provides strong evidence that this motif is a receptor binding specificity determinant of fibroblast growth factors.

The fibroblast growth factor (FGF)[1] ligand−receptor system comprises nine genetically distinct polypeptide mitogens (Miyamoto et al., 1993) and four tyrosine kinase transmembrane receptors (Johnson & Williams, 1993). Heparan sulfate proteoglycans (HSPG) play a pivotal role in expression of the biological activity in that an interaction of the growth factor with HSPG is an obligatory step in the binding of FGF to its receptor (Yayon et al., 1991; Ornitz et al., 1992). Further, HSPG interacts independently of FGF ligand with a specific sequence in the extracellular domain of the FGF receptor (Kan et al., 1993). An additional level of complexity in the FGF receptor (FGFR) multigene family is created by alternative mRNA splicing which results in combinatorial variants that are functionally different (Johnson et al., 1991; Givol & Yayon, 1992). The ectodomain of FGFR is organized into three immunoglobulin-like (Ig-like) domains. The first N-terminal domain is not required for ligand binding, whereas the second and third domains are important for binding heparin (Kan et al., 1993), ligand binding, and differential recognition of the members of the FGF ligand family (Werner et al., 1992; Yayon et al., 1992; Zimmer et al., 1993; Cheon et al., 1994).

The three-dimensional structures of FGF-1 and FGF-2 reveal a backbone structure composed of 12 antiparallel β-strands connected by solvent-exposed loop and β-turn structures arranged to form a β-barrel that is closed at one end (Zhu et al., 1991; Ago et al., 1991; Eriksson et al., 1991; Zhang et al., 1991). Of note is that the folding pattern observed for the FGF structures is strikingly similar to that of the interleukins IL-1α and IL-1β. Significant progress has been made in understanding the role and identity of the residues involved in the molecular recognition of heparin by FGF-2 (Thompson et al., 1994; Li et al., 1994), but a parallel advancement in defining the residues that participate in receptor binding is lacking. A putative receptor binding domain in the primary sequence of FGF-2 was identified on the basis of studies using a peptide fragment of FGF-2 comprising residues 115−124 that exhibits properties consistent with those of a receptor binding peptide (Baird et al., 1988). The finding that phosphorylation of threonine 121 in this sequence enhances the binding of FGF-2 to its receptor (Feige & Baird, 1989) and that the three-dimensional structure of FGF-2 revealed within this sequence a solvent-exposed loop (residues 118−122) between the ninth and tenth β-strands provided further evidence to support the notion that this region is involved in receptor binding (Zhu et al., 1991; Eriksson et al., 1991; Zhang et al., 1991). The precise nature of FGF−receptor−heparin interactions and the protein residues involved have yet to be elucidated.

To probe FGF structure−function, we carried out mutagenesis of FGF-2 that involved the exchange of loop structures in the protein with the corresponding motifs selected from other members of the FGF family and the structural homologue IL-1β. This approach is designed to

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[1] Abbreviations: FGF, fibroblast growth factor; HSPG, heparan sulfate proteoglycan; IL-1β, interleukin-1β; FGF-2LA, FGF-2 containing a loop from acidic FGF (FGF-1); FGF-2LI, FGF-2 containing a loop from interleukin-1β; Ig-like, immunoglobulin-like; DMEM, Dulbecco's modified Eagle's medium; AP, alkaline phosphatase; FRAP, fibroblast growth factor receptor alkaline phosphatase; FGFR, fibroblast growth factor receptor; KGFR, keratinocyte growth factor receptor; BHK cells, baby hamster kidney cells; EC_{50}, half-maximal stimulatory concentration.

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identify discrete functional domains and minimize the impact on the structural integrity of both the protein and the transplanted loop by selecting the donor and host proteins from the same structural class. Thus, to investigate the role of the FGF-2 loop sequence, 118−122, in receptor binding we replaced the β9−β10 loop in FGF-2 with the corresponding loop sequences from FGF-1 and IL-1β. Binding studies, using soluble receptor systems that can distinguish between different FGF ligands, and chemical cross-linking of radiolabeled ligands revealed that the β9−β10 loop in FGF-2 and the corresponding loop sequence in FGF-1 function to define receptor binding specificity.

EXPERIMENTAL PROCEDURES

Materials. Sodium heparin from porcine intestinal mucosa was obtained from Hepar Industries (Franklin, OH). FGF-7 was obtained from UBI (Lake Placid, NY). $Na^{125}I$ was purchased from Amersham (Buckinghamshire, England). FGFs were iodinated by using the chloramine T method, as described (Yayon et al., 1992). Specific activities of the preparations were (1.2−1.7) × 10^5 cpm/ng FGF, and the preparations were kept for up to 3 weeks at −70 °C. Dulbecco's modified Eagle's medium (DMEM; 1 g of glucose/L), calf serum, fetal calf serum, penicillin, and streptomycin were obtained from Cellgro. Saline containing 0.05% trypsin, 0.01 M sodium phosphate, and 0.02% EDTA (STV) was obtained from Cellgro. Tissue culture dishes were from Falcon Labware Division, Becton Dickinson (Oxnard, CA). Four-well tissue culture plates were from Nunc (Rosklide, Denmark). All other reagents were of the highest quality commercially available.

Construction of Human FGF-2LA and FGF-2LI. Cassette directed mutagenesis was used to construct the FGF-2LI and FGF-2LA loop replacement mutants. The gene encoding human $Glu^{3,5}$ FGF-2 (Seddon et al., 1991) was cloned into the T7 expression vector pET-3a(M13), a derivative of pET-3a (Rosenberg et al., 1987), between restriction sites *Nde*I and *Bam*H1. Two unique restriction endonuclease sites, *Bst*BI and *Spl*I, were introduced into the gene in such a way as to produce no change in the encoded amino acids (i.e., silent mutations) at positions that flank the codons encoding the segment Ser117−Trp123 of FGF-2. Replacement of residues Arg118-Lys119-Tyr120-Thr121-Ser122 of FGF-2 with the human sequence Ala-Gln-Phe-Pro-Asn from the corresponding loop of the structural analogue IL-1β (115−119) (Table 1) was as follows. The plasmid DNA was subjected to *Bst*BI and *Spl*I digestion, and the larger DNA fragment was isolated using agarose gel electrophoresis. The DNA fragment was ligated, using T_4 DNA ligase, to a double-stranded DNA obtained by annealing two synthetic oligonucleotides, 5′-CGAACGATTG GAATCTAATA ACTACAATAC GTACCGGTCT GCGCAGTTTC CTAACTGGTA TGTGGCACTT AAGC-3′ and 5′-GTACGCTTAA GTGCCACATA CCAGTTAGGA AACTGCGCAG ACCGGTACGT ATTGTAGTTA TTAGATTCCA ATCGTT-3′, that contain termini compatible with those generated by *Bst*BI and *Spl*I digestion. The ligation product was used to transform *Escherichia coli* (strain DH5α) cells. The desired mutant plasmid was selected for on the basis of susceptibility to cleavage at the newly introduced Afl2 restriction site (underlined). Replacement of the segment Arg118−Ser122 of FGF-2 with the bovine sequence Lys-Lys-His-Ala-Glu-Lys-His, corresponding to the surface loop (115−121) of the structural analogue FGF-1 (Table 1), was accomplished as described above using the following annealed synthetic mutagenic oligonucleotides: 5′-CGAACGATTG GAATCTAATA ACTACAATAC GTACCGGTCT AAAAAGCATG CTGAAAAACA CTGGTATGTG GCACTTAAGC-3′ and 5′-GTACGCTTAA GTGCCACATA CCAGTGTTTT TCAGCATGCT TTTTAGACCG GTACGTATTG TAGTTATTAG ATTCCAATCG TT-3′. The desired mutant plasmid was selected for on the basis of susceptibility to cleavage at the newly introduced *Sph*I restriction site (underlined).

Table 1: Structural Alignment[a] of the β9−β10 Loops in FGF-1, FGF-2, and IL-1β

	110	115	120	125	130
FGF-1	ENHYNTYIS	**KKHAEKH**	WFVGLKKNG		
	110	115	120	125	130
FGF-2	SNNYNTYRS	**RKY..TS**	WYVALKRTG		
	110	115	120	125	
IL-1β	.NNKLEFES	**AQF..PN**	WYISTSQAE		

[a] Numbering for FGF-1 and FGF-2 is from amino acid residue 1 deduced from the cDNA sequence encoding the 155-residue form, and that for IL-1β is from residue 1 of the mature 153-residue polypeptide.

Expression and Purification of FGF-2LA and FGF-2LI Mutants. Following sequence verification, the plasmids containing the genes encoding the FGF-2LA and FGF-2LI loop mutants were transformed into competent *E. coli* BL21 plys S and cultured at 37 °C in Luria broth containing 50 μg/mL ampicillin and 30 μg/mL chloramphenicol until an absorbance at 600 nm of 0.4 was reached. Expression of the recombinant protein was induced by the addition of 2 mM isopropyl thiogalactoside for 2 h at 37 °C. Cells from a 1-L culture were harvested by centrifugation, resuspended in 30 mL of 50 mM Tris-HCl, pH 7.5, containing 0.1 mM EDTA and 0.6 M NaCl, and disrupted by treatment with lysozyme (10 μg/mL) for 20 min at 4 °C followed by sonication (6 × 30 s pulses). The lysates were clarified by centrifugation (10000*g*; 20 min), and the supernatant solutions incubated with 5 mL of hydrated heparin Sepharose (Pharmacia/LKB) at 4 °C for 1 h with constant rotation. The resin was isolated by filtration on a 0.8-μm filter apparatus (Nalgene), washed extensively with 10 mM Tris-HCl, pH 7.4, containing 0.6 M NaCl and bound protein eluted with Tris buffer containing 3 M NaCl (25 mL). The 3 M NaCl eluent was diluted 6-fold with Tris buffer and loaded onto a TSK heparin 5PW column (0.21 × 15 cm; The Nest Group, Southborough, MA), and the column was developed using a linear gradient (0.6−2 M NaCl) in 90 min at a flow rate of 3 mL/min.

FGF-2LA and FGF-2LI were purified to apparent homogeneity and obtained in a final yield of about 8 mg/L of bacterial culture. The proteins exhibited a single 18-kD a silver-stained band by SDS−PAGE and a single peak by reverse-phase HPLC. N-Terminal sequence analysis gave the sequences AEGEITTLPA (87%) and MAEGEITTLPA (13%), indicating that a small fraction of the proteins retained the N-terminal methionyl residue (introduced for the expression of the mature form of the protein). Amino acid analyses were consistent with the predicted composition of the mutant proteins. Protein concentrations were determined by amino acid analysis.

Soluble FGF Receptor Construction and Transfection. Soluble FGF receptor proteins were constructed by cloning of the extracellular region of murine FGFR-1 (IIIc variant), FGFR-2 (IIIc variant), or FGFR-2IIIb (KGFR) into the alkaline phosphatase-tag expression vector, which encodes for a secreted form of placental alkaline phosphatase (AP) (Yayon et al., 1992). The FGF receptor alkaline phosphatase (FRAP) plasmids were cotransfected into NIH 3T3 cells by electroporation with a selectable neomycin resistance gene. Colonies were selected in G418 (600 μg/mL) and screened for secreted AP enzyme activity in the conditioned medium. Clones of each receptor which produced a high level of AP activity (2−4 units/min/mL) were then used to produce conditioned medium for binding assays.

Receptor Binding Assays. Components of the soluble receptor binding reaction mixture included FRAP conditioned medium (0.24 OD units/min), 2 ng/mL [^{125}I]FGFs, and 1 μg/mL heparin. The FGF−heparin−FRAP ternary complex was immunoprecipitated with 20 μL of a 1:1 slurry of anti-AP monoclonal antibodies coupled to protein A−Sepharose (Ornitz et al., 1992). All components were mixed at room temperature. The total volume was adjusted to 200 μL by addition of DMEM containing 0.1% bovine serum albumin. Binding was allowed to proceed for 1−2 h at 24 °C, after which time the bound receptor complex or the ligand was recovered by centrifugation at 4 °C (10 s at 2000g). The pelleted material was washed twice with 500 μL of an ice-cold buffer containing Hepes (20 mM), NaCl (150 mM), glycerol (10%), and Triton X-100 (1%). [^{125}I]FGF binding was quantitated by counting of the samples in a gamma counter (LKB).

High-Affinity Binding of FGF Proteins to NIH 3T3 Cells. Receptor binding was performed as described (Moscatelli, 1987). Briefly, FGF bound to heparan sulfate low-affinity sites was released from the cell surface by a 5-min incubation with an ice-cold solution containing 1.6 M NaCl and 20 mM HEPES, pH 7.4, and the amount of radioactivity released was determined with a gamma counter. FGF bound to high-affinity receptors was dissociated by a 2 M NaCl (20 mM acetate buffer, pH 4.0) extraction, and the released labeled FGF was quantitated.

Cross-Linking of FGF Proteins to FGF Receptors. Chemical cross-linking experiments were carried out at room temperature in a volume of 50 μL in siliconized 1.5-mL microcentrifuge tubes. The reaction mixtures contained FGF receptor immobilized to anti-AP monoclonal antibodies coupled to protein A−Sepharose, 1 μg/mL heparin, 2 ng/mL [^{125}I]FGF, 20 mM phosphate buffer (pH 7.4), and 140 mM NaCl. After a 90-min incubation, 1 mL of a solution of disuccinimidyl suberate (Pierce), dissolved in dimethyl sulfoxide, was added to give a final concentration of 0.15 mM, and the mixture was incubated for an additional 30 min. The reaction was quenched by addition of 1 mL of 200 mM ethanolamine-HCl (pH 8.0) for 30 min. The reaction mixtures were diluted 1:1 with 2× SDS−polyacrylamide gel electrophoresis loading buffer and electrophoresed on an SDS−12% polyacrylamide gel. FGF cross-linked to the FGF receptor was detected by autoradiography on Kodak XAR film.

RESULTS

The sequence alignments for FGF-2, FGF-1, and IL-1β derived from their three-dimensional structures in comparison

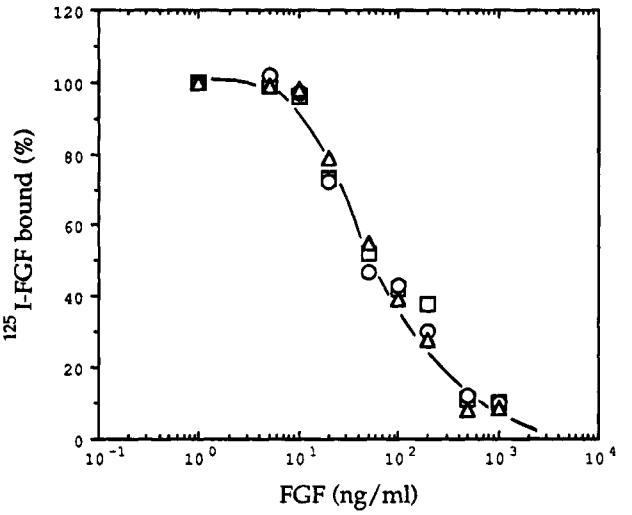

FIGURE 1: Binding of FGF-2, FGF-2LA, and FGF-2LI to NIH 3T3 cells. NIH 3T3 cells were incubated with 2 ng/mL [^{125}I]FGF-2 (○), [^{125}I]FGF-2LA (□), or [^{125}I]FGF-2LI (△) in 0.25 mL of DMEM containing 0.1% BSA for 90 min at 4 °C. Cell surface receptor-associated radioactivity was determined as described under Experimental Procedures. Nonspecific binding was determined in the presence of a 100-fold excess of unlabeled FGF-2 and did not exceed 15% of the total binding and was subtracted. The line drawn is for illustration purposes only.

to other members of the FGF family are shown in Table 1. The affinities of the mutants for immobilized heparin were found to be identical to those of wild-type FGF-2, and the mutants required about 1.4 M NaCl to effect their elution from a TSK-heparin column. The biological properties of the proteins were assessed by their ability to induce proliferation of cultured adult bovine aortic arch endothelial cells as described by Gospodarowicz et al. (1984). The potency of the FGF-2LA protein was identical to that of the wild-type protein (EC_{50}, 0.3 ng/mL), whereas the FGF-2LI mutant was about 3-fold less active. In a DNA synthesis assay for IL-1β activity using D10G.4 cells, FGF-2LI and FGF-2 were not mitogenic, nor did they antagonize the mitogenic activity of IL-1β (A. P. Seddon, unpublished data).

Binding of FGF Proteins to Cell Surface Receptors. Since FGF-1 and FGF-2 bind to FGFR-1 present on BHK and NIH 3T3 cells with equal affinity, we predicted that the FGF-2 mutant containing the surface loop from FGF-1 would bind with the same affinity as FGF-2, whereas the FGF-2 mutant containing the IL-1β loop would exhibit a significantly diminished affinity, on the basis of the suggested involvement of this region of FGF-2 in receptor binding (Baird et al., 1988). Figure 1 shows the results of competition binding experiments on NIH 3T3 cells that express FGFR-1 using radioiodinated versions of FGF-2, FGF-2LA, and FGF-2LI as a function of added unlabeled FGF. As expected, FGF-2LA displayed the same receptor binding affinity as the wild-type protein. Surprisingly, FGF-2LI also exhibited the same receptor binding affinity as FGF-2. Binding curves for FGF-2 and the mutant proteins using BHK cells as a source of FGF receptor were identical to those obtained with NIH 3T3 cells (A. P. Seddon, unpublished data). The data show that the competition binding curves for FGF-2LA and FGF-2LI are identical to that for FGF-2 and demonstrate that the loop exchanges in FGF-2 have no impact on the receptor binding affinities of the proteins to FGFR-1 present on BHK and NIH 3T3 cells.

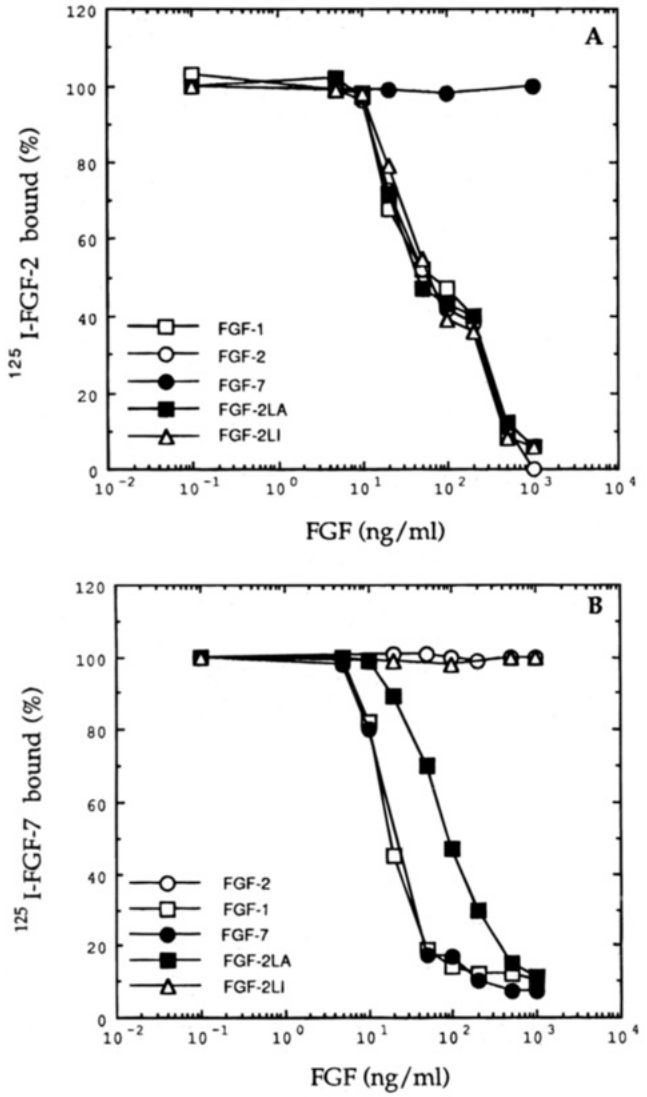

FIGURE 2: Displacement binding analysis of FGFs bound to soluble receptors by various FGFs and FGF-2 mutants. The binding of various FGFs to immobilized FGF receptors was determined by displacement analysis of (A) ^{125}I-labeled FGF-2 from FGFR-1 by various unlabeled FGFs and (B) ^{125}I-labeled FGF-7 from KGFR by various unlabeled FGFs as described under Experimental Procedures.

Binding of FGF Proteins to Soluble Receptors. The position of the surface loop in FGF-2 coincides with a variable-sequence region in the FGF family of proteins where insertions occur (Miyamoto et al., 1993). We reasoned that this region may be involved in determining ligand−receptor binding specificity since it is known that many of the nine members of the FGF family exhibit different receptor-type binding specificity profiles. The binding profiles of the loop mutants, FGF-2LA and FGF-2LI, to FGFR-1 and KGFR, which does not bind FGF-2, were determined. Panels A and B of Figure 2 show the competition binding curves to soluble versions of FGFR-1 and KGFR for FGF-1, FGF-2, FGF-7, and the FGF-2 loop mutants following the displacement of radioiodinated FGF-2 and FGF-7 from the soluble receptors, respectively. FGF-1, FGF-2, FGF-2LA, and FGF-2LI bind equally well to FGFR-1, but no binding of FGF-7 was detected over the range examined (Figure 2A). The binding profiles of various FGF proteins to KGFR (Figure 2B) show that KGF and FGF-1 bind with equal affinity and that FGF-2 and the FGF-2LI mutant do not bind to this receptor type;

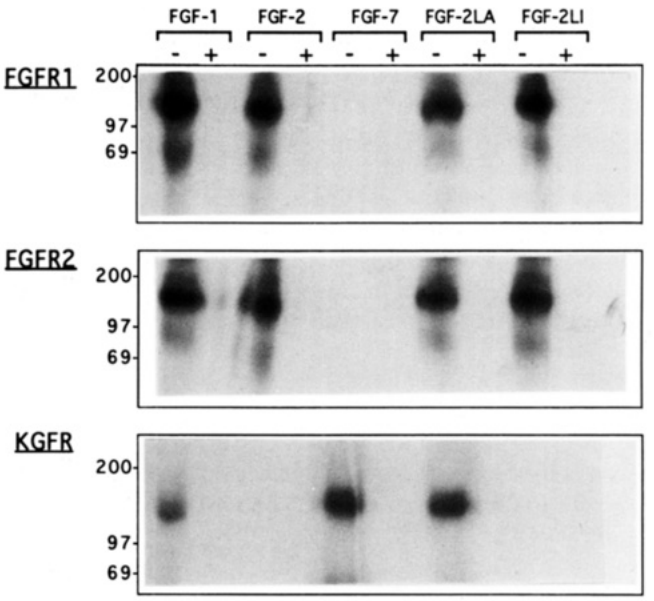

FIGURE 3: Cross-linking of FGFs to soluble receptors. Immobilized soluble FGFR-1, FGFR-2, and KGFR were incubated with [^{125}I]-labeled FGFs as indicated, in the presence (+) or the absence (−) of a 100-fold excess of the corresponding unlabeled FGF. Receptor-bound FGF was cross-linked to the receptor by treatment with disuccinimidyl suberate, and the products were resolved by SDS−polyacrylamide gel electrophoresis as described under Experimental Procedures. Radiolabeled protein bands were detected by autoradiography.

however, when the loop in FGF-2 was replaced with the corresponding loop from FGF-1, the affinity of the FGF-2LA mutant for KGFR was only about 5 times less than that for FGF-7 or FGF-1. That the receptor binding profile of FGF-2LA now resembles that for FGF-1 and not FGF-2, the host protein, is consistent with the involvement of this surface motif in determining receptor−ligand specificity.

Cross-Linking of FGF Proteins to Different FGF Receptors. The binding specificities of radiolabeled FGF proteins to various soluble FGF receptors were confirmed by chemical cross-linking of the factors to the receptors in the presence and absence of an excess of the unlabeled FGF proteins. Alternative transcripts encode KGFR and FGFR-2; the latter binds FGF-1 and FGF-2, whereas KGFR binds only KGF and FGF-1 with high affinity. Figure 3 shows the cross-linking patterns for FGF-1, FGF-2, KGF, FGF-2LA, and FGF-2LI to FGFR-1, FGFR-2, and KGFR soluble receptors. FGF-1, FGF-2, FGF-2LA, and FGF-2LI bind to FGFR-1, and addition of an excess of the appropriate unlabeled FGF abolishes cross-linking of the corresponding [^{125}I]FGF to the receptor. Cross-linking of [^{125}I]KGF to FGFR-1 was not detected. A cross-linking profile identical to that for FGFR-1 was obtained using soluble FGFR-2. The cross-linking profile for KGFR, however, reveals that only FGF-1, KGF, and the FGF-2LA mutant bind to this receptor and that FGF-2 and FGF-2LI do not bind. Thus, results from chemical cross-linking of the different FGF proteins to different receptor proteins are in accord with the binding data (Figures 1 and 2).

DISCUSSION

Surface loops and β-turns are important secondary structural elements and play a crucial role in orientation of binding residues in ligand recognition and biological function (Rose

et al., 1985). Such structures are attractive candidates for protein engineering in that they are short units of primary sequence, constrained by interactions with adjacent secondary structures, and they assume a conformation determined by the sequence of the loop or turn and the geometry of the host site. The feasibility of transferring a β-turn structure as a "structural cassette" or "module" from one protein context to another with retention of the parent conformation and function when substituted in the host protein was demonstrated by Hynes et al. (1989). The present study demonstrates the use of the approach to probe the function of loop structures by replacement of a loop in the FGF-2 "host" protein with the corresponding structures selected from another member of the FGF ligand family and a structurally related protein.

The present work defines a structural motif in the primary sequence of FGF-2 and FGF-1 involved in the differential recognition of members of the FGF receptor family. This receptor specificity site resides in a loop connecting the ninth and tenth β-strands. Transfer of the $\beta9-\beta10$ loop from FGF-1 and IL-1β to FGF-2 apparently does not result in significant structural perturbations, since the mutant proteins retain wild-type-like endothelial cell proliferation activity. Also, the loop structure does not seem to contribute markedly to the stabilization of the heparin−FGF−FGFR-1 ternary complex, as the binding of FGF-2LI and FGF-2LA to heparin and cultured cells expressing FGFR-1 was unchanged. Inspection of the aligned primary structures for members of the FGF ligand family revealed considerable variation in the size of this motif and prompted us to examine receptor binding specificity of the mutants compared to that of the parent FGF protein.

At the level of the receptor an understanding of the role of alternative splicing in modulating receptor−ligand interactions came from comparing the ligand binding specificities of the two Ig-like domain FGFR-1 variants that contain IIIb- and IIIc-type sequences in the C-terminal half of the third Ig-like domain (Werner et al., 1992) and FGFR-2 containing the IIIc-type sequence to KGFR (IIIb sequence variant) and chimeras that contain IIIc and IIIb alternative sequences, respectively (Yayon et al., 1992). These studies provided evidence to show that (a) FGF-1 binds to FGFR-1 and FGFR-2 variants that contain either IIIb or IIIc sequences; (b) FGF-2 binds with highest affinity to only the IIIc variant forms of the receptors; and (c) FGF-7, keratinocyte growth factor, binds only to the IIIb variant of FGFR-2. Thus, the C-terminal region of the third Ig-like domain of the receptor is a structural element that defines the binding specificity of different FGF family members.

We found that replacement of a five amino acid residue turn in FGF-2 (net charge of +2) with the corresponding five-residue element from IL-1β, which contains no charged amino acid side chains and has a similar α-carbon backbone conformation (Eriksson et al., 1991), had no impact on the receptor binding specificity profile of the host molecule. A dramatic switch, however, in receptor binding specificity or receptor recognition capability of FGF-2, to mimic that of FGF-1, occurred with the transfer of the seven-residue turn sequence (net charge of +4) from FGF-1 into FGF-2. Our results indicate that the $\beta9-\beta10$ loop is a critical component that participates in the determination of receptor binding specificity in FGF-1 and FGF-2. Although it is not immediately apparent how the residues in the loop participate in receptor recognition and subsequent binding, the loop exchanges suggest that specificity of receptor recognition may be primarily related to size and conformation of the motif rather than presentation of charge alone. The observation that FGF-2LA binds to KGFR nearly as tightly as does FGF-1 suggests not only that the guest loop in FGF-2LA is presented to KGFR in a configuration highly similar to that which it assumes in FGF-1 but also that other components of the receptor binding domain of FGF-2, presumably located at positions surrounding the $\beta9-\beta10$ loop, are topographically similar to those of FGF-1 in order for FGF-2LA to exhibit such a high affinity for KGFR. On the basis of the observation that FGF-1 binds to all receptor isoforms, the interaction of $\beta9-10$ loop in FGF-1 with the receptors may be passive or even non-interactive. For FGF-2 and potentially for other FGF ligands the corresponding loop elements may also be passive with receptor types that bind ligand, but for receptors that reject an FGF the loop motif may serve to misalign binding surfaces through an unfavorable interaction. The situation may be far more complicated when the size of the loop is increased, as is the case for FGF-7 and several other FGF family members. Here the increased size of the insert may favor a structure that is not under the tight constraints imposed by the supporting β-strands that exist for small interconnecting sequences, such that a direct binding interaction between ligand and receptor occurs. Although it is tempting to generalize this finding to include other members of the FGF ligand family, further investigation of this motif in the context of other FGF ligands is required and is currently underway.

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