Biochemical and Biophysical Research Communications **283,** 791–797 (2001)
doi:10.1006/bbrc.2001.4850, available online at http://www.idealibrary.com on IDEAL®

Evidence That the Intracellular Domain of FGF Receptor 2IIIb Affects Contact of the Ectodomain with Two FGF7 Ligands

Fumiyuki Uematsu,* Jun-Hyeog Jang,†,[1] Mikio Kan,*,[2] Fen Wang,* Yongde Luo,* and Wallace L. McKeehan†,[3]

†*Department of Biochemistry and Biophysics, Texas A&M University and Center for Cancer Biology and Nutrition, and* **Institute of Biosciences and Technology, Texas A&M University System Health Science Center, Houston, Texas 77030-3303*

Received April 3, 2001

Models of the oligomeric FGF signaling complex, including those derived from crystal structures, vary in stoichiometry and arrangement of the three subunits comprised of heparin/heparan sulfate chains, FGFR tyrosine kinase and activating FGF. Here, using covalent affinity crosslinking of radiolabeled FGF7 to binary complexes of FGFR2IIIb and heparin, we show that two molecules of FGF7 contact each FGFR2IIIb. This supports models that propose a dimeric complex of two units with stoichiometry 1 FGF:1 FGFR in which each FGF contacts both FGFR. The bivalent FGF7 contact was dependent on the full-length amino terminus of FGF7α and the intracellular domain of FGFR2IIIb extending through the juxtamembrane domain and the β1 and β2 strands of the kinase which is required for ATP binding. We propose that the differences in crosslinking report differences in relationships among subunits in the ectodomain of the complex that are affected by the amino terminus of FGF and the FGFR intracellular domain. From this, we suggest the corollary that conformational relationships among subunits in the ectodomain are transmitted to the intracellular and ATP binding domains during activation of the complex.

Key Words: **tyrosine kinases; oligomeric complexes; heparan sulfate; transmembrane signaling.**

The FGF signal transduction system is a ubiquitous intrinsic mediator of cell-to-cell communication that determines cell behavior in development and adult homeostasis (1). The large family consists of 23 genetically distinct FGF homologues and a plethora of isoforms generated by alternate splicing from four transmembrane receptor (FGFR) tyrosine kinase genes (1). Signaling occurs from oligomeric complexes of FGF, FGFR kinase and the heparan sulfate chains of pericellular matrix proteoglycan (1). The structure of the oligomeric complex, its assembly, and mode of activation is unclear. Generally, it is agreed that activation of the complex promotes a *trans*-phosphorylation event between intracellular domains that allows access of substrates to the kinases (1). From both biochemical and structural data of individual components, diverse and fundamentally different models of the FGFR complex with different stoichiometry and interactions between FGF, FGFR kinase and heparan sulfate have been proposed (1). Recently, the issue has become further clouded by emergence of fundamentally different models based on crystal structures derived from essentially the same FGF, FGFR ectodomain and artificial heparin-derived oligosaccharide (2, 3). One structure indicates a symmetric complex of 2 FGF:2 heparin chains:2 FGFR kinases comprised of two units of a ternary complex of FGF:heparin chain:FGFR (2). Each FGF and heparin chain contacts and bridges both FGFR ectodomains. The other structure indicates an asymmetric complex of 2 FGF:1 heparin chain:2 FGFR comprised of one ternary complex of FGF:heparin chain:FGFR ectodomain and one complex of only FGF:FGFR (3). Each FGF and the heparin chain contact a single FGFR. Simply the heparin chain that binds asymmetrically across the two FGFs bridges the FGF/FGFR units. In this report, we show by analysis of covalently crosslinked complexes resulting from the

[1] Current address: Laboratory of Cell Biology, Cancer Research Center and Cancer Research Institute, Seoul National University College of Medicine, Seoul, Korea 110744.

[2] Current address: Central Research Laboratories, Zeria Pharmaceutical Co., Ltd., 2512-1 Oshikiri, Kohnan-machi, Ohsato-Gun, Saitama, Japan 360-0111.

[3] To whom correspondence should be addressed at Institute of Biosciences and Technology, Texas A&M University System Health Science Center, 2121 W. Holcombe Boulevard, Houston, TX 77030-3303. Fax: 713-677-7512. E-mail: wmckeeha@ibt.tamu.edu.

0006-291X/01 $35.00

binding of radiolabeled FGF7 to a binary complex of heparin and FGFR2IIIb that two FGF7 molecules contact each FGFR2βIIIb. This supports the former model of the oligomeric FGFR complex described above (2). Moreover, we show that the crosslinked complex that correlates with a stoichiometry of 2 FGF7:1 FGFR2IIIb requires the intact NH2-terminal sequence of FGF7 and is also determined by the intracellular domain of FGFR2IIIb. Since the FGFR intracellular domain affects the apparent bivalent interaction of FGF7 with FGFR2IIIb ectodomains, we suggest the corollary that the bivalent interaction of FGF with both FGFRIIIb ectodomains in dimers can be transmitted to the intracellular domain of the FGFR kinase during activation of the complex.

MATERIALS AND METHODS

Expression and purification of recombinant FGF7. The 18-kDa FGF7β (^{54}Ser-FGF7) isoform was prepared in bacteria by expression of the coding sequence for *S. japonicum* glutathione-S-transferase (GST) fused in frame with that of FGF7 beginning at Ala-30 in vector pGEX-2T (Amersham Pharmacia Biotech, Piscataway, NJ) as described (4). Treatment with trypsin while immobilized to heparin–Sepharose and subsequent elution by salt gradient results in pure FGF7β exhibiting the ^{54}Ser N-terminus (Fig. 1A). Preparations containing the 23-kDa isoform with the amino-terminal sequence gly-ser-^{30}ala-cys, hereafter called FGF7α, were prepared by the same procedure in bacteria, but generated from the thrombin cut site placed between GST and the ala-30 of FGF7. The preparation contains variable amounts of FGF7β due to cleavage in the bacterial extract at ser-30. Identity of products was evaluated by SDS–polyacrylamide gel electrophoresis and amino-terminal sequence of electroeluted bands.

Oligonucleotide primers used in FGFR2IIIb constructs. Oligonucleotides indicated below were purchased from Integrated DNA Technologies, Inc (Coralville, IA). Restriction sites are underlined and nucleotides that are not in the coding sequence for FGFR2IIIb or FGF7 are in lowercase: 5R2αC, 5′ CCA GAG GAT CCA AAG TGG GAA TTC 3′; 3R2αC(+), 5′ cgt cta gaa GGC CCC CAG GAG GTT GAT GAT GT 3′; 3R2αC(−), 5′ cgt cta gaT GGC GTC ATC TTT CAA CAT CTT CA 3′; 3R2αC2, 5′ cgt cta gaG TCT GTC CTT ATC GAT TCC CAC CG 3′; 5R2AP, 5′ CCG GCT GTG CAC AAG CTG ACC AAG 3′; 3R2JM2, 5′ cgt cta gaG TGT CGT TAT CCT CAC CAG TGG GGT 3′; 3R2d, 5′ cgt cta gaG ATC CTC TGG CAA CTC GTA CTC AG 3′.

Construction and expression of recombinant FGFR2IIIb cDNAs. Constructions of FGFR2IIIb employed in the study are shown in Fig. 1B. cDNA coding for the full-length rat FGFR2βIIIb isoform (5, 6) was used as a template in the polymerase chain reaction (PCR). The fusion protein FGFR2IIIbGST, in which glutathione S-transferase (GST) began at Asp-383 of FGFR2IIIb, was prepared as described (7). The constructs JM and $\Delta\delta$K were made by amplifying sequences downstream of the *Apa*LI site in the intracellular region of FGFR2IIIb by PCR. Primers employed were 5R2AP and 3R2JM2 for JM and 5R2AP and 3R2d for $\Delta\delta$K. After treatment with *Apa*LI and *Xba*I these fragments were ligated with the *Pst*I–*Apa*LI fragment of FGFR2IIIb cDNA, and then cloned into the insect cell expression vector pVL1392 (Invitrogen, Carlsbad, CA). The αC2, αC(−) and αC(+) were made by amplifying sequences downstream of the BamH1 site in the intracellular region by the PCR with FGFR2IIIb cDNA as the template. Primers used were 5R2C and 3R2α2 for αC2, 5R2C and 3R2αC(−) for αC(−), and 5R2C and 3R2αC(+) for αC(+). After treatment with *Bam*HI and *Xba*I the fragments were ligated with the *Pst*I–*Bam*HI fragment of FGFR2IIIb cDNA, and then cloned into vector pVL1392 (Invitrogen). Recombinant baculoviruses bearing the FGFR2IIIb constructs were prepared and expressed as described below.

Binding and covalent affinity crosslinking of ^{125}I-FGF. FGF1 (bovine recombinant), FGF2 (human recombinant, purchased from Upstate Biotechnology, Lake Placid, NY), FGF7α, and FGF7β were iodinated, reactivated by reduction and purified to a specific activity of 2 to 5 $\times$ 10^5 cpm/ng (8). Recombinant baculovirus encoding FGFR2IIIb constructs were prepared, and viruses were transfected into Sf9 insect cells (8, 9). Where indicated, FGFR2IIIbGST was extracted with detergent and immobilized onto GSH Sepharose beads (Amersham Pharmacia Biotech). Cells expressing FGFR2IIIb on the surface or immobilized FGFR2IIIbGST were collected by centrifugation, washed and used in binding assays. All binding experiments were performed by introducing radiolabeled FGF to divalent cation-dependent binary complexes of FGFR2IIIb and heparin (9) prepared by pre-incubation of cells or immobilized FGFR2IIIb with heparin and subsequent removal of soluble heparin (7). Analysis of the binding and covalent affinity crosslinked complexes was performed as described (7, 8). Except where otherwise indicated, the radiolabeled complexes were subjected to 7.5% SDS–polyacrylamide gel electrophoreses (SDS–PAGE) and then subsequent autoradiography.

RESULTS AND DISCUSSION

A crosslinked species with apparent stoichiometry of 2 FGF7:1 FGFR2IIIb requires the full-length amino terminus of FGF7α and Ig modules II and III in the ectodomain of FGFR2IIIb. In experiments designed to identify structural subdomains required for high affinity binding of amino terminal variants of FGF7 to the ectodomain of fusions with GST on the intracellular domain of FGFR2IIIb (Fig. 1), we observed a unique radiolabeled FGFR2IIIb product with preparations containing FGF7α (7). The unique crosslinked species had an apparent molecular weight that was about 24 kDa higher than that observed for the expected complex of one ^{125}I-labeled FGF7 and one FGFR2IIIb-GST (Fig. 2A). The higher molecular weight complex was not apparent when radiolabeled FGF1 beginning at residue Pro-25 and FGF2 beginning at residue Pro-9 was employed. Appearance of the higher molecular weight band was dependent on the presence of 23-kDa FGF7α with the intact amino terminus. When pure radiolabeled 18-kDa FGF7β beginning at Ser-54 was employed, only the single band with apparent molecular mass that correlated with a complex with stoichiometry of 1 FGF7:1 FGFR2IIIb-GST was apparent (Fig. 2A). The binding of both FGF7α and FGF7β was strictly dependent on the presence of heparin.

We investigated whether the crosslinked complex with apparent stoichiometry 2 FGF7α:1 FGFR2IIIb-GST was due to a crosslinked dimer or higher order aggregate of FGF7α that was crosslinked to an FGFR2IIIb ectodomain. Using 15% SDS–PAGE gels, we analyzed a portion of samples from the same binding assays after exposure to crosslinker. Subsequent autoradiography revealed only the presence of the FGF7α and FGF7β monomers present in the fraction of bound radiolabeled FGF7 that was not crosslinked to

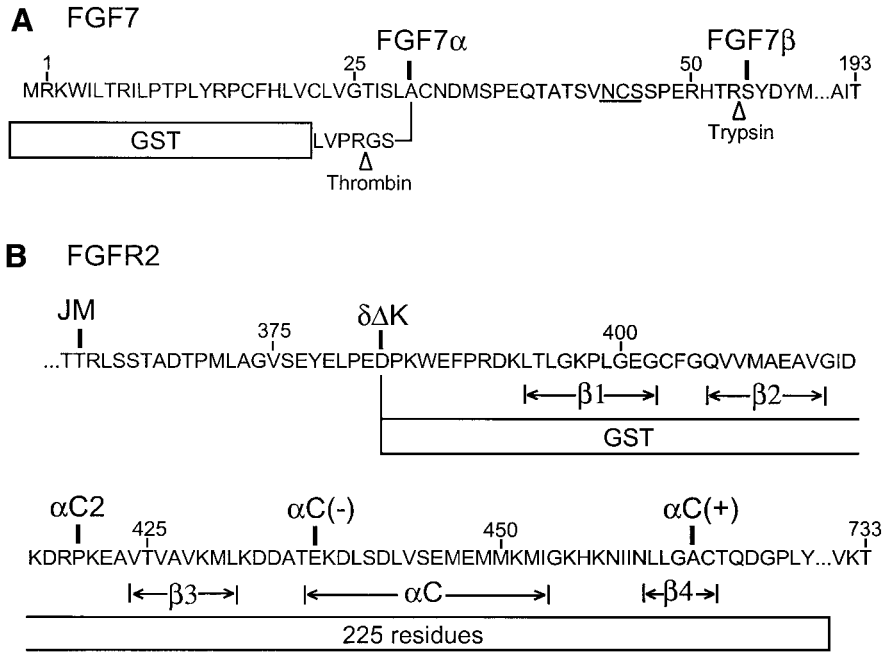

FIG. 1. (A) Isoforms and constructs of rat recombinant FGF7. The full translation product containing the secretory signal, FGF7α, and FGF7β is indicated. Heparin-bound recombinant gly-ser-^{30}ala-FGF7α and ^{54}ser-FGF7β were generated by treatment of the indicated GST-FGF7 with thrombin or trypsin, which cuts at the respective sites indicated. Recombinant FGF7α corresponds to a native form of FGF7 beginning at Ala-30, but without the glycosylation (underlined) (23). (B) Constructs and structural subdomains of the FGFR2IIIb intracellular domain. Expression products described in the text are indicated at the point of truncation. All recombinant expression products included the secretory signal, the acid box, Ig modules II and IIIb, the transmembrane domain, and the indicated lengths of the intracellular domain. Numbering of amino acids in FGFR2IIIb is based on full-length rat FGFR2IIIb cDNA.

the FGFR2IIIb (Fig. 2B), a profile which was similar to the radiolabeled ligands before introduction into binding assays (Fig. 2C). No labeled material could be detected that would indicate dimers of either isoform of FGF7 even when gels were exposed for prolonged periods. We cannot eliminate the possibility that dimers of FGF7α that fail to covalently crosslink with each other are bound to and crosslink to one FGFR2IIIb ectodomain, however, increasing evidence from several sources suggests that FGF binds to FGFR as a monomer. These include diverse models of the FGF-FGFR complex derived from crystal structures (2, 3, 10, 11), FGFR-binding residues are masked in crystal structures of FGF9 containing dimeric forms (12, 13) and dimers and higher order aggregates of FGF1 and FGF2 appear less active (14, our unpublished results). We demonstrated previously (7) that the higher molecular weight species with apparent 2 FGF7α:1 FGFR2IIIb-GST stoichiometry requires Ig modules II and III and the interloop sequence that is required to form stable dimers of the FGFR ectodomain (15). The kinetics of competition binding experiments among different FGFs with radiolabeled FGF7α suggest that monomeric FGFR comprised of two or three Ig modules can accommodate only one FGF even though complexes of 1 FGF7:1 FGFR2 can form from isolated individual Ig modules (1, 7, our unpublished results). Analysis of stoichiometry in solution and in FGF–FGFR crystal structures (3, 10, 11) also show that monomers are bound to FGFR. Therefore, we deduced that the higher molecular weight crosslinked species with stoichiometry 2 FGF7α:1 FGFR2IIIb most likely arises from a symmetric dimer of units of 1 FGF7α:1 FGFR2IIIb in which each FGF7α is capable of crosslinking to either of the FGFR2IIIb within the dimer (Fig. 3).

The crosslinked species with apparent stoichiometry of 2 FGF7α:1 FGFR2IIIb requires the FGFR2IIIb intracellular sequence through β1 and β2 strands of the kinase domain. Similar to the FGFR2βIIIb-GST construct, FGFR2βIIIb exhibiting a full-length intracellular domain exhibited the two radiolabeled species differing by about the molecular mass of FGF7α (Fig. 4A). The higher molecular weight band only appeared when FGF7α was employed in the binding assays. As reported previously (16), the binding of FGF1, FGF2, FGF7α, and FGF7β were dependent on the presence of heparin. Surprisingly, when FGFR2βIIIb was truncated beginning at thr-61 in the juxtamembrane domain (construct FGFR2IIIbJM, Fig. 1B), only the band that corresponded with the 1 FGF7:1 FGFR2βIIIb complex was apparent (Fig. 4B). Moreover, formation of the single crosslinked species with FGF1, FGF2, FGF7α, and FGF7β was independent on heparin.

We then determined more precisely the impact of the intracellular sequence on appearance of the higher mo-

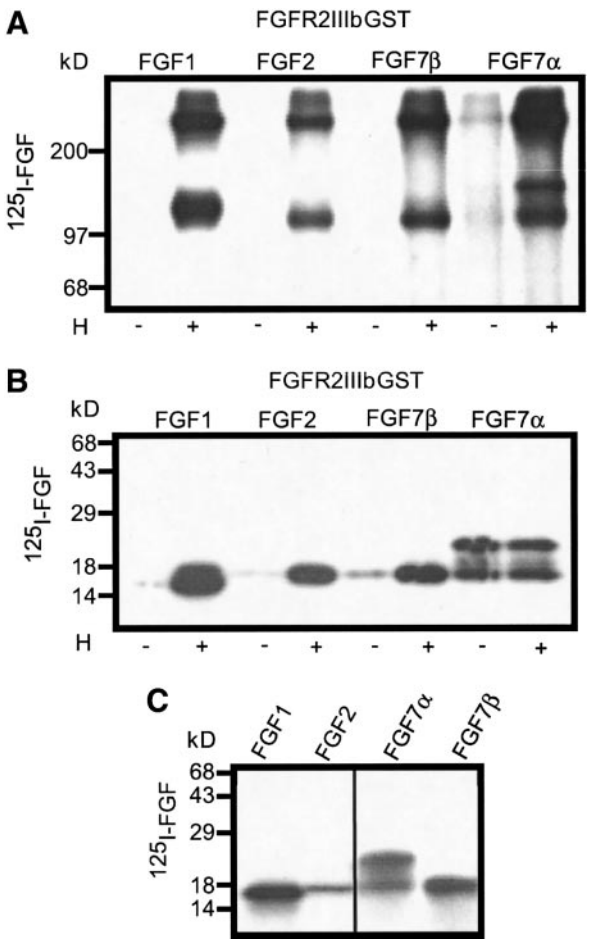

FIG. 2. ^{125}I-FGF binding to FGFR2IIIb fused with GST at asp-383. (A, B) Bound and crosslinked radiolabeled material recovered from binding assays. The recombinant FGFR2IIIb construct fused with GST at asp-383 (Fig. 1) was expressed in insect cells, extracted, and immobilized onto GSH-Sepharose beads. The FGFR2IIIb-GST was preincubated with (+) or without (−) 1 μg/ml heparin (H), unbound heparin was removed, and the resulting complex was incubated with the indicated ^{125}I-labeled FGF prior to covalent crosslinking with disuccinimidyl suberate (DSS). Samples were split into two equal parts and analyzed either by autoradiography of (A) 7.5% SDS–PAGE gels which measured crosslinked complexes with FGFR or (B) 15% SDS–PAGE gels which measured free FGF both bound to FGFR and nonspecifically bound that failed to crosslink to FGFR. (C) Radiolabeled FGF1, FGF2, FGF7α, and FGF7β prior to binding assays. The indicated radiolabeled FGFs were also analyzed on 15% SDS–PAGE gels.

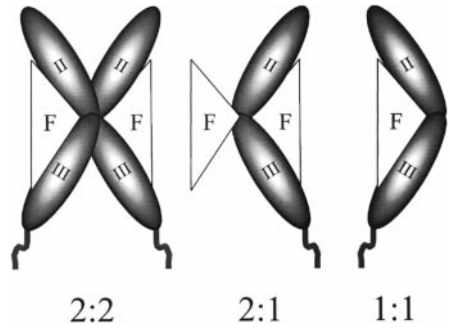

FIG. 3. Potential crosslinked species from a symmetrical dimer with stoichiometry 2 FGF7α:2 FGFR2IIIb in which FGF is bivalent. A symmetrical 2:2 dimer of two FGFRβ monomers each occupied by one FGF without the HS chains is indicated (1, 2, 10, 11). A single crosslinking event between FGF and an FGFR results in the 1 FGF:1 FGFR species. A single crosslinking event from each FGF to the same FGFR results in the 2:1 species. Appearance of a 2:2 crosslinked species may arise from multiple crosslinking events from a single FGF to different FGFR or between two FGFR in addition to the single FGF–FGFR links to yield complexes that do not penetrate the gels employed in this study.

lecular weight crosslinked species and eliminated the possibility that the two bands were due to systematic heterogeneity of FGFR2IIIb. We examined the binding of FGF7α and FGF7β to a series of FGFR2IIIb constructs truncated at different positions within the intracellular domain shown in Fig. 1B. Both of the crosslinked species emerging from preparations of FGF7α were apparent from constructs exhibiting the amino terminus of the kinase domain through β-strands 1 and 2 (17). The higher molecular weight band was lost by truncation at asp-383 (mutant FGFR2IIIb$\delta\Delta$K) just upstream of β1 and β2 in the kinase domain sequence (Fig. 5A). The higher molecular weight species resulting from crosslinking of FGF7α to each of the slightly different sized truncates through FGFR2IIIbαC2 differed by about the molecular weight of one FGF7 (Table 1) while all constructs from FGF7β exhibited only one band (Fig. 5B). On average, the difference between the lower molecular

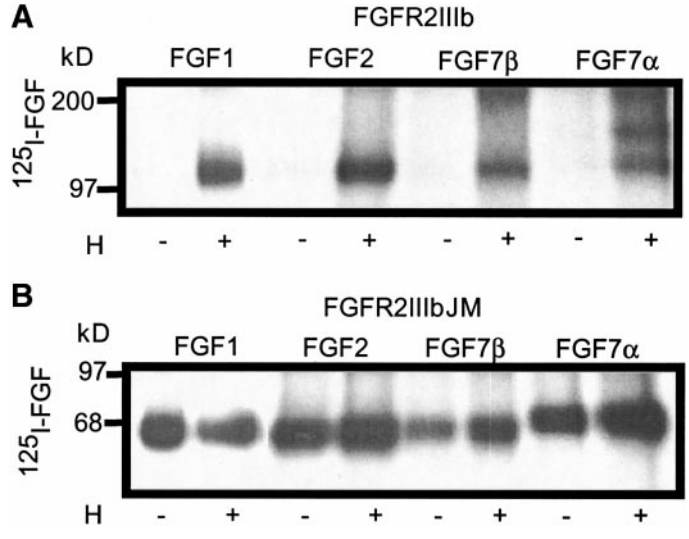

FIG. 4. Crosslinked complexes of radiolabeled FGF full-length FGFR2IIIb and a variant truncated within the intracellular juxtamembrane sequence. The binding and crosslinking of the indicated FGF to full-length FGFR2IIIb (A) and FGFR2IIIb truncated at thr-361 (B) expressed on the surface of insect cells was analyzed. Binding assays with (+) and without (−) a preincubation with 10 μg/ml heparin (H) and subsequent removal of unbound heparin were performed.

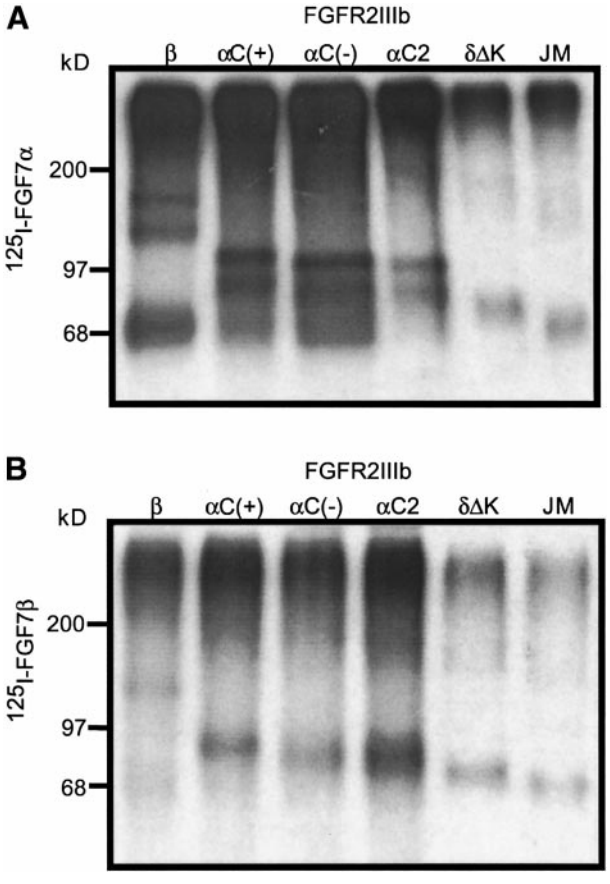

FIG. 5. Crosslinked complexes of FGF7α and FGF7β to variants of FGFR2IIIb in the intracellular domain. Crosslinked complexes from the binding of FGF7α (A) and FGF7β (B) to the indicated constructs (Fig. 1B) on the surface of insect cells after preincubation with 10 μg/ml heparin were analyzed.

species of the two from FGF7α and the single band from FGF7β was equal to the differences in molecular weight between the two FGF7 isoforms (Table 1). A radiolabeled natural truncate of about 70 kDa which arises internally from proteolysis of full length FGFR2IIIb at a site in the juxtamembrane sequence (18) was near the size of the engineered truncation in mutant FGFR2IIIbJM. This internally generated truncate also exhibited only one crosslinked species with FGF7α in contrast to its full-length parent FGFR2IIIb in the same cells (Fig. 5A, lane 1). In separate experiments not shown here, we demonstrated that isoform FGFR2αIIIb, which contains a third Ig-like module amino terminal to the two modules of FGFR2βIIIb, also exhibits two crosslinked species with specifically FGF7α which differ by the molecular weight of FGF7α. These results strongly suggest that the higher molecular weight species that results with specifically FGF7α is a complex of 2 FGF7 molecules crosslinked to 1 FGFR2IIIb and not due to variations in molecular weight of the FGFR2IIIb constructs. The intracellular domain affects a conformation required for covalent affinity crosslinking of a second FGF7α to the extracellular domain of FGFR2IIIb. Moreover, the intracellular domain also affects the requirement for heparin for the binding of FGF7 to the ectodomain, independent of the amino terminus of FGF7.

CONCLUSIONS

Here we used covalent affinity crosslinking of radiolabeled FGF7 to binary complexes of heparin and FGFR2IIIb as an indirect reporter of relationships among the three components in the ectodomain of the FGFR complex. We conclude that the amino terminus of FGF7 and the intracellular domain of FGFR2IIIb affect the conformational relationships among subunits in the extracellular domain of the oligomeric complex of FGF7-heparin-FGFR2IIIb. If the intracellular domain affects relationships in the ectodomain monitored by the crosslinking, then it follows that those relationships in the ectodomain among FGF isoforms, heparin/heparan sulfate and the FGFR kinase ectodomain communicate with the intracellular domain. This is consistent with a conformational model of repression of activity and activation of an FGFR complex in which conformational relationships, not simply the proximity of components, is the rate-limiting factor in overall activity of the complex (1, 7, 9).

TABLE 1

Apparent Molecular Weights of Radiolabeled Crosslinked Complexes of ^{125}I-FGF7 to FGFR2IIIb Variants (kDa)

FGFR2IIIb	GST	β	αC(+)	αC(−)	αC2	δΔK	JM
FGF7α	132 ± 5	140 ± 5	110 ± 6	105 ± 4	103 ± 6		
	Δ24	Δ22	Δ20	Δ17	Δ18		
	108 ± 3	118 ± 5	90 ± 5	88 ± 6	85 ± 5	79 ± 4	73 ± 6
	Δ5	Δ3	Δ6	Δ6	Δ5	Δ5	Δ3
FGF7β	103 ± 7	115 ± 6	84 ± 5	82 ± 8	80 ± 7	74 ± 5	70 ± 6

Note. Values were estimated from the middle of bands against a plot of log molecular weight versus migration distance with four standards. The ± values indicated are the width of bands. Δ values indicate the difference in kDa between the bands above and below the value. The molecular weights of FGF7α and FGF7β calculated from amino acids are 18.9 and 16.1 kDa, respectively. Mean molecular weights (± width of band) estimated from Fig. 2C were 23.4 (±0.8) and 17.5 (±0.6) kDa, respectively.

Recently, two fundamentally different models of the FGF–heparin–FGFR oligomeric complex derived from crystal structures have been reported (2, 3, 10, 11). Our results support the symmetric structure comprised of two 1 FGF:1 FGFR units in which FGF molecules are bivalent, each contacts both FGFR in the dimer, and each FGF and FGFR are bridged by a separate heparin or heparan sulfate chain (2, 10, 11). This model is similar in principle to conformational models of the FGFR complex that we have proposed that accommodate most structural and functional data in the FGF literature (1, 7).

It is unclear whether the covalent affinity crosslinking results shown here suggest a general interaction of FGF ligands or are unique to FGF7 and FGFR2IIIb. On the one hand, the amino-terminal sequence of FGF7α may present novel crosslinking groups that allow detection of a general interaction of FGF ligands including FGF7β with two FGFR through production of the stable 2 FGF:1 FGFR-labeled species. On the other hand, the results may indicate impact of the amino-terminal sequence of FGFα on specific conformational relationships among subunits in the FGFR complex, suggest that FGF7α and FGF7β bind differently into the oligomeric complex, and thus exhibit different functions. The 23 members of the FGF polypeptide family are distinguished by unique amino-terminal sequences whose functions are poorly understood.

Finally, we showed that the native FGFR2IIIb intracellular sequence that impacts the crosslinking pattern in the ectodomain of the complex spans the juxtamembrane and β1 and β2 strands of the kinase domain. This includes highly conserved sequences required for ATP binding and activity of the kinase. The juxtamembrane domain of FGFR has been implicated in the interaction with intracellular substrates (19–22). It is likely that the role of the β1 and β2 strands of the kinase on the crosslinking pattern are structural rather than sequence-specific since GST can mimic effect of the β1 and β2 sequence domain. In separate experiments, we have shown that the first 50 residues of GST also confer the two-band pattern and that several other artificial sequences up to 35 residues in length fused at asp-383 cannot mimic the GST and native FGFR2 sequences. Since the intracellular juxtamembrane and sequences required for ATP binding affect conformation in the ectodomain reported by the crosslinking pattern, we suggest the corollary that FGF binding and conformational change in the ectodomain may be transmitted to the juxtamembrane domain and ATP binding site to activate the FGFR complex.

ACKNOWLEDGMENTS

This work was supported by Public Health Service Grants DK35310, DK47039, and CA59971.

REFERENCES

1. McKeehan, W. L., Wang, F., and Kan, M. (1998) The heparan sulfate-fibroblast growth factor family: Diversity of structure and function. *Prog. Nucleic Acid Res. Mol. Biol.* **59,** 135–176.
2. Schlessinger, J., Plotnikov, A. N., Ibrahimi, O. A., Eliseenkova, A. V., Yeh, B. K., Yayon, A., Linhardt, R. J., and Mohammadi, M. (2000) Crystal structure of a ternary FGF-FGFR-heparin complex reveals a dual role for heparin in FGFR binding and dimerization. *Mol. Cell* **6,** 743–750.
3. Pellegrini, L., Burke, D. F., von Delft, F., Mulloy, B., and Blundell, T. L. (2000) Crystal structure of fibroblast growth factor receptor ectodomain bound to ligand and heparin. *Nature* **407,** 1029–1034.
4. Luo, Y., Lu, W., Mohamedali, K. A., Jang, J. H., Jones, R. B., Gabriel, J. L., Kan, M., and McKeehan, W. L. (1998) The glycine box: A determinant of specificity for fibroblast growth factor. *Biochemistry* **37,** 16506–16515.
5. Yan, G., Wang, F., Fukabori, Y., Sussman, D., Hou, J., and McKeehan, W. L. (1992) Expression and transforming activity of a variant of the heparin-binding fibroblast growth factor receptor (flg) gene resulting from splicing of the alpha exon at an alternate 3′-acceptor site. *Biochem. Biophys. Res. Commun.* **183,** 423–430.
6. Wang, F., Kan, M., Xu, J., Yan, G., and McKeehan, W. L. (1995) Ligand-specific structural domains in the fibroblast growth factor receptor. *J. Biol. Chem.* **270,** 10222–10230.
7. Uematsu, F., Kan, M., Wang, F., Jang, J. H., Luo, Y., and McKeehan, W. L. (2000) Ligand binding properties of binary complexes of heparin and immunoglobulin-like modules of FGF receptor 2. *Biochem. Biophys. Res. Commun.* **272,** 830–836, doi: 10.1006/bbrc.2000.2872.
8. Kan, M., Wu, X., Wang, F., and McKeehan, W. L. (1999) Specificity for fibroblast growth factors determined by heparan sulfate in a binary complex with the receptor kinase. *J. Biol. Chem.* **274,** 15947–52.
9. Kan, M., Wang, F., To, B., Gabriel, J. L., and McKeehan, W. L. (1996) Divalent cations and heparin/heparan sulfate cooperate to control assembly and activity of the fibroblast growth factor receptor complex. *J. Biol. Chem.* **271,** 26143–26148.
10. Stauber, D. J., DiGabriele, A. D., and Hendrickson, W. A. (2000) Structural interactions of fibroblast growth factor receptor with its ligands. *Proc. Natl. Acad. Sci. USA* **97,** 49–54.
11. Plotnikov, A. N., Schlessinger, J., Hubbard, S. R., and Mohammadi, M. (1999) Structural basis for FGF receptor dimerization and activation. *Cell* **98,** 641–650.
12. Plotnikov, A. N., Eliseenkova, A. V., Ibrahimi, O. A., Shriver, Z., Sasisekharan, R., Lemmon, M. A., and Mohammadi, M. (2001) Crystal structure of fibroblast growth factor 9 reveals regions implicated in dimerization and autoinhibition. *J. Biol. Chem.* **276,** 4322–4329.
13. Hecht, H. J., Adar, R., Hofmann, B., Bogin, O., Weich, H., and Yayon, A. (2001) Structure of fibroblast growth factor 9 shows a symmetric dimer with unique receptor- and heparin-binding interfaces. *Acta Crystallogr. D Biol. Crystallogr.* **1,** 378–384.
14. Engleka, K. A., and Maciag, T. (1992) Inactivation of human fibroblast growth factor-1 (FGF-1) activity by interaction with copper ions involves FGF-1 dimer formation induced by copper-catalyzed oxidation. *J. Biol. Chem.* **267,** 11307–11315.
15. Wang, F., Kan, M., McKeehan, K., Jang, J. H., Feng, S., and McKeehan, W. L. (1997) A homeo-interaction sequence in the ectodomain of the fibroblast growth factor receptor. *J. Biol. Chem.* **272,** 23887–23895.
16. Jang, J. H., Wang, F., and Kan, M. (1997) Heparan sulfate is required for interaction and activation of the epithelial cell fi-

broblast growth factor receptor-2IIIb with stromal-derived fibroblast growth factor-7. *In Vitro Cell. Dev. Biol. Animal* **33,** 819–824.

17. Mohammadi, M., Schlessinger, J., and Hubbard, S. R. (1996) Structure of the FGF receptor tyrosine kinase domain reveals a novel autoinhibitory mechanism. *Cell* **86,** 577–587.
18. Xu, J., Nakahara, M., Crabb, J. W., Shi, E., Matuo, Y., Fraser, M., Kan, M., Hou, J., and McKeehan, W. L. (1992) Expression and immunochemical analysis of rat and human fibroblast growth factor receptor (flg) isoforms. *J. Biol. Chem*. **267,** 17792–17803.
19. Gillespie, L. L., Chen, G., and Paterno, G. D. (1995) Cloning of a fibroblast growth factor receptor 1 splice variant from *Xenopus* embryos that lacks a protein kinase C site important for the regulation of receptor activity. *J. Biol. Chem*. **270,** 22758–22763.
20. Lin, H. Y., Xu, J., Ischenko, I., Ornitz, D. M., Halegoua, S., and Hayman, M. J. (1998) Identification of the cytoplasmic regions of fibroblast growth factor (FGF) receptor 1 which play important roles in induction of neurite outgrowth in PC12 cells by FGF-1. *Mol. Cell. Biol*. **18,** 3762–3770.
21. Xu, H., Lee, K. W., and Goldfarb, M. (1998) Novel recognition motif on fibroblast growth factor receptor mediates direct association and activation of SNT adapter proteins. *J. Biol. Chem*. **273,** 17987–17990.
22. Ong, S. H., Guy, G. R., Hadari, Y. R., Laks, S., Gotoh, N., Schlessinger, J., and Lax, I. (2000) FRS2 proteins recruit intracellular signaling pathways by binding to diverse targets on fibroblast growth factor and nerve growth factor receptors. *Mol. Cell. Biol*. **20,** 979–989.
23. Rubin, J. S., Osada, H., Finch, P. W., Taylor, W. G., Rudikoff, S., and Aaronson, S. A. (1989) Purification and characterization of a newly identified growth factor specific for epithelial cells. *Proc. Natl. Acad. Sci. USA* **86,** 802–806.