Heparin-induced Self-association of Fibroblast Growth Factor-2
EVIDENCE FOR TWO OLIGOMERIZATION PROCESSES*  

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Fibroblast growth factor-2 (FGF-2), a potent angiogenic factor, requires heparin for dimerization and activation of the FGF receptor tyrosine kinase. The binding of multiple fibroblast growth factors by heparin may be necessary for dimerization of the FGF receptor. Analytical ultracentrifugation of FGF-2 in the presence of heparin or heparan sulfate (HS) shows that both an inactive heparin octasaccharide and an active heparin-like disaccharide induce fibroblast growth factor-2 self-association. Analysis of the data indicates that the heparin octasaccharide induces a monomer-dimer-trimer assembly of FGF-2 while the disaccharide induces a monomer-dimer equilibrium. Evidence is presented indicating that the dimer conformation induced by the heparin octasaccharide is a side by side dimer with the FGF-2 molecules cis to the heparin, while the disaccharide-induced dimer is a head to head dimer in which FGF-2 molecules are trans to the ligand. These results, combined with previous studies, support the model that formation of a specific side by side heparin-induced FGF-2 dimer is required for activation of the FGF receptor.

Fibroblast growth factor-2 (FGF-2) is a cytokine whose biological activity is dependent on heparin or heparan sulfate (HS, collectively) (1). The binding of FGF-2 and heparin to the FGF receptor (FGFR) activates a signal transduction cascade, eventually stimulating cell proliferation and differentiation. Cells that are activated by FGF-2 require two classes of receptors: low-affinity receptors which are heparan sulfate proteoglycans (HSPGs) and high-affinity receptors which are transmembrane receptor tyrosine kinases (2). The heparin or HSPG low-affinity receptors are thought to bind to FGF initially and present FGF to the FGF receptor (3), leading to a ternary FGF receptors complex that can then activate the FGF signal transduction pathway (1). It has been shown that activation of the FGF signal transduction pathway occurs upon dimerization of the FGF tyrosine kinase receptor and transphosphorylation of each kinase domain (4).

The role of heparin in activating the FGF signaling pathway is unclear. Heparin has been shown to interact with the FGF receptor directly (5) and may facilitate the formation of a high-affinity FGF-FGFR complex by bridging the two proteins and binding to each (6). Another model is that heparin may activate the FGFR by binding several FGFs, thereby recruiting two cell-surface FGFRs and promoting transphosphorylation of the FGFR kinase domains (1).

Heparin has been shown to bind multiple FGF molecules (1, 7–9). These studies have shown that one FGF molecule can bind per every 4–5 saccharide units in heparin. Heparin-induced oligomerization of FGF-1 and FGF-2 has been observed by cross-linking studies (1, 9, 10); cross-linking has also confirmed dimerization of FGF-2 by an active nonsulfated oligosaccharide analog (11). These results support the model that heparin binds multiple FGFs to activate the receptor, but the question remains whether heparin is merely binding multiple FGFs nonspecifically along the long heparin chain, or whether heparin is involved in forming a specific dimer conformation of FGF-2 that is required for activation of the FGFR.

Recently, the crystal structures of two biologically active, nonsulfated oligosaccharides bound to FGF-2 were determined (11). These structures both showed three binding sites (labeled 1, 2, and 2') for the oligosaccharides. Site 1 corresponded to the well characterized high-affinity heparin-binding site (7, 12, 15, 22). Sites 2 and 2', previously undescribed, brought together two FGF-2 molecules by simultaneously binding to a single saccharide ligand, suggesting that the biological activity of these small nonsulfated compounds may be due to ligand-mediated dimerization of FGF-2. This hypothesis was supported by a cross-linking experiment showing FGF-2 dimerization in the presence of one of these ligands (11). Venkataraman and colleagues (13) compared this FGF-2/nonsulfated oligosaccharide structure with six crystal structures of apo-FGF-2 and found that all of the structures exhibited identical side by side positioning of the FGF-2 monomers. The authors proposed that apo-FGF-2 has a weak dimerization interface close to the site 2/side 2' region that promotes formation of a side by side dimer.

Such a dimer would allow the binding of a single heparin chain of 8–10 saccharide units (corresponding to what has been reported as the shortest active heparin fragment (1, 14)) to the high-affinity heparin-binding site (site 1) contributed by each FGF-2 molecule (illustrated in Fig. 4C). In their modeling studies, Venkataraman et al. (13) showed that the binding of a heparin octasaccharide to a side by side FGF-2 dimer (with both FGF-2 molecules cis to the heparin ligand) would stabilize...
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the dimer by increasing the protein contact surface area from 761 Å² in the apo-dimer to 2036 Å² in the heparin-bound dimer.

To better understand the role of heparin and heparin-like compounds in oligomerizing FGF-2, we have used analytical ultracentrifugation to analyze the association state of FGF-2 in the presence of the shortest reported biologically active heparin fragment, an octasaccharide (HS-8) (1). For comparison, we also analyzed the association state of FGF-2 in the presence of sucrose octasulfate (SOS), an inactive sulfated disaccharide which binds to FGF-2 (20) but is too small to bridge the high-affinity heparin-binding sites of a putative side by side dimer.

EXPERIMENTAL PROCEDURES

Sample Preparation—FGF-2 was generously provided by J. Abraham (Scios Nova) as a stock solution of 8.3 mg/ml in 20 mM sodium citrate, pH 5.0, 1 mM EDTA, and 9% sucrose. Heparin octasaccharide fragment HS-8 (derived from heparin by nitrous acid depolymerization) in phosphate-buffered saline was a gift from C. Svahn (Pharmacia). The sodium salt of SOS was a gift from Bukh Meditcon. Concentration of protein was determined spectrophotometrically (A280 = 0.964 (16)). For all titration experiments involving HS-8, FGF-2 was dialyzed extensively against a buffer containing 20 mM sodium citrate, pH 6.9, 1 mM EDTA, 5 mM β-mercaptoethanol (β-ME), and 140 mM NaCl. The solutions of FGF-2 and HS-8 were then diluted into “sedimentation buffer” containing 20 mM sodium citrate, pH 6.9, 1 mM EDTA, and 5 mM β-ME, to achieve a final NaCl concentration of 20 mM. For the study of the effect of salt, FGF-2 was dialyzed against sedimentation buffer, and NaCl was added to appropriate levels as indicated under “Results.” For titration experiments involving SOS, the stock solution of FGF-2 was initially diluted 100-fold into the sedimentation buffer and recentered to approximately 10 mg/ml by ultrafiltration (Amicon centricron 10). However, because interference with sucrose was observed (see “Results”), later titration and salt effect experiments were carried out with FGF-2 extensively dialyzed in sedimentation buffer.

Analytical Ultracentrifugation—Sedimentation equilibrium experiments were conducted using a Beckman XL-A Optima analytical ultracentrifuge equipped with an absorbance optical system. Runs were carried out at 20,000, 25,000, and 30,000 rpm, at 20 °C. Six-channel, charcoal-filled epon cassettes with quartz windows were used in a four-hole Ti-60A titanium rotor. Sample volumes in the two inner sample wells of each cassettes were 100 μl, and the outer well contained 90 μl. Each sample well contained 10 μl (inner two wells) or 30 μl (outer well) of FC46 (Beckman), an inert fluorocarbon oil that displaces the sample solution in the outermost region of the well, thereby making the data that can be collected. The reference wells were filled with 125 μl of the appropriate buffer. Radial scans at 280 nm were collected between 5.9 and 7.2 cm as the average of five measurements, with a step size of 0.001 cm. The samples were allowed to achieve sedimentation equilibrium over the course of 14–20 h, depending on the rotor speed. Samples were considered at equilibrium when sequential scans 2 h apart were superimposable. The partial specific volume of FGF-2 was calculated as 0.734 g/ml based on its amino acid composition, using values from Ref. 17. The contribution of ligand to the partial specific volume of FGF-2 was considered to be negligible, since the partial specific volumes of sugars are similar to proteins (17) and since the mass of FGF-2 is substantially larger than the masses of the ligands used. The buffer density was determined to be 0.996 g/ml, calculated from tables in Ref. 17 and the density of β-ME.

Nonlinear Least-squares Analysis—The raw data files were edited using the program REEDIT (D. Yphantis) and then analyzed by global nonlinear least-squares analysis using the program NONLIN (18). All terms from tables in Ref. 17 and the density of β-ME were done as described in Ref. 11. Dimer band intensities were quantified by scanning densitometry. The density of each dimer band was corrected by subtracting the density observed in the absence of saccharide; this corrects for nonspecific random cross-linking in the absence of saccharide.

RESULTS

Each experiment consisted of a control sample of apo-FGF-2 as well as samples of FGF-2 in the presence of a saccharide ligand. Apo-FGF-2 consistently sedimented as a single species whose molecular weight clearly corresponded to that of a monomer (calculated Mr = 17,100) (data not shown). To determine whether heparin-derived compounds induce FGF-2 self-association in solution, sedimentation experiments with FGF-2 in the presence of HS-8 or sucrose octasulfate were carried out.

Sedimentation equilibrium experiments were carried out with FGF-2 and various concentrations of saccharide ligand. The first set of experiments included solutions of 11.9 μM FGF-2 with 5.95, 11.9, 17.9, 23.8, 47.6, 119, or 238 μM HS-8. The data at HS-8 concentrations below 47.6 μM fit best to a monomer-dimer-tetramer association scheme, as shown in Fig. 1, A and B. Fits to monomer-dimer-trimer or monomer-dimer-trimer-tetramer assembly models were not as good. The data for HS-8 concentrations of 47.6 μM or above fit best to a monomer-dimer-dimer association, as shown in Fig. 1B. The highest levels of association were seen at 5.95 μM HS-8; the monomer-dimer dissociation constant at this HS-8 concentration was 4.42 μM (K2 in Fig. 4B), and the dimer-tetramer stepwise dissociation constant was 109 μM (K3 in Fig. 4B). The dimerization associ-
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**Fig. 1.** HS-8 induced monomer-dimer-tetramer assembly of FGF-2. A, sedimentation equilibrium data for 11.9 μM FGF-2 with 11.9 μM HS-8, at 30,000 rpm, in 20 mM sodium citrate pH 6.9, 1 mM EDTA, 5 mM β-ME, 20 °C. The continuous line through the data points represents the best fit to the data, a monomer-dimer-tetramer assembly model. The dashed lines indicate the concentrations of the monomer, dimer, and tetramer species. Inset, a plot of the residual differences of observed data minus fitted data. B, dependence of dimerization and tetramerization on HS-8 concentration. Log K4 (M⁻³) and log K2 (M⁻¹) are plotted versus log [HS-8] (M). Data points correspond to best fits obtained from global analysis of data from a single sample, at speeds of 30,000, 25,000, and 20,000 rpm. Black triangles represent values of log K4, and black circles represent values of log K2. At HS-8 concentrations of 47.6 μM or above, fits to monomer-dimer-tetramer equilibria showed negligible levels of tetramerization and therefore K4 values are not shown for these solutions. Error bars correspond to the error values generated by the program NONLIN. The box at the top of the plot shows a schematic representation of the assembly models that gave the best fits to the data. M refers to monomer, D to dimer, and T to tetramer.

... tion constants showed a slight decrease with increasing HS-8 concentration, as shown in Fig. 1B. The tetramerization (monomer-tetramer) association constants K4 also decreased with increasing HS-8 concentration, and tetramerization fell off to undetectable levels at HS-8 concentrations of 47.6 μM or above. The negative slope implies that although HS-8 is required for FGF-2 dimerization under these conditions, higher concentrations of HS-8 interfere with FGF-2 assembly. This is presumably due to saturation of the heparin-binding site on FGF-2, which would shift the equilibrium toward FGF-2 monomers, each bound to a single HS-8 molecule.

To compare the action of HS-8 on FGF-2 with that of another sulfated saccharide, sedimentation experiments were also carried out with FGF-2 and SOS. These experiments included solutions of 11.9, 17.9, 23.8, 119, 238, 476, 714, or 952 μM SOS in 11.9, 17.9, 23.8, or 47.6 μM FGF-2. All solutions containing FGF-2 and SOS exhibited an FGF-2 monomer-dimer equilibrium, as shown in Fig. 2A for a representative set of data. However, the data from the FGF-2/SOS experiments showed no clear trend with increasing concentrations of SOS, which we suspected was due to interference from residual sucrose in the FGF-2 storage buffer (sucrose may interfere with binding of SOS, as another nonsulfated oligosaccharide (11) has been shown to bind to the same site to which SOS binds (15)). Therefore, in a second set of experiments, solutions were prepared after the FGF-2 had been extensively dialyzed in sedimentation buffer (20 mM sodium citrate, pH 6.9, 1 mM EDTA, 5 mM β-ME), and sedimentation equilibrium experiments were carried out. As before, all the data fit best to a monomer-dimer equilibrium. The highest levels of association were seen at 952 μM SOS; the monomer-dimer dissociation constant at this SOS concentration was 92.91 μM. The dimerization constant increased with increasing SOS in an approximately linear fashion between 119 and 952 μM SOS, as shown in Fig. 2B. This shows that binding of SOS in this range of concentration is positively linked to FGF-2 dimerization. However, in the lower range (below 119 μM) of SOS concentration studied, although fits to monomer-dimer equilibria were excellent, no clear trend in the value of the association constant was observed (data not shown). Fig. 2B shows that the data for the sucrose-containing solutions with SOS concentrations equal to or higher than 476 μM (open squares) coincided with the data from the sucrose-free solutions. High concentrations of SOS would appear to compete effectively with sucrose for binding to FGF-2.

The observation of a SOS-induced dimer was intriguing and could be interpreted as a mere artifact of the relatively high FGF-2 concentrations used. Therefore, FGF-2 dimerization in the presence of SOS at two concentrations (4 and 12 μM) was monitored by cross-linking, a more sensitive technique. FGF-2 concentration was 585 nM, well below the concentrations used for the sedimentation equilibrium experiments. A control experiment with HS-8 was also performed. Dimerization of FGF-2 was clearly observed in the presence of both SOS and HS-8. Addition of 4.0 or 12 μM SOS reproducibly increased...
FGF-2 dimerization by 22.2 or 22.3%, respectively, over nonspecific cross-linking (in the absence of saccharide), and addition of equivalent concentrations of HS-8 increased FGF-2 dimerization by 49.9 and 25.5% over nonspecific levels. Results for HS-8 were similar to previously published data for a 16-saccharide heparin fragment (11). These results verify that both SOS and HS-8 are capable of inducing dimerization of FGF-2 at lower concentrations of FGF-2 than can be reliably observed using most biophysical techniques, and in a different buffer system (22 mM NaPO$_4$, pH 7.4, 150 mM NaCl) than that used in the sedimentation studies.

To further characterize this system, sedimentation experiments were also carried out with varying salt concentrations. Solutions of 17.9 $\mu$m FGF-2 with 17.9 $\mu$m HS-8 were prepared in sedimentation buffer containing increasing concentrations of NaCl (0, 0.05, 0.1, 0.25, 0.5, and 1 $\mu$M NaCl). Solutions of FGF-2/HS-8 at low salt concentrations (0 and 0.05 M NaCl) showed the monomer-dimer-tetramer equilibrium described above. However, at NaCl concentrations above 50 mM, the equilibrium shifted to a monomer-dimer equilibrium and subsequently to a single monomeric species at 1 $\mu$M NaCl, as shown in Fig. 3A. Sedimentation experiments were also carried out for FGF-2/SOS complexes; solutions of 17.9 $\mu$m FGF-2 and 17.9 $\mu$m SOS were prepared in sedimentation buffer containing 0, 0.05, 0.1, 0.25, 0.5, and 1 $\mu$M NaCl. A monomer-dimer equilibrium was observed at NaCl concentrations between 0 and 0.25 $\mu$M NaCl, but at NaCl concentrations of 0.5 and 1 $\mu$M NaCl, only a single monomeric species was observed, as shown in Fig. 3B.

**DISCUSSION**

The sedimentation equilibrium experiments described above reveal that HS-8 induces a monomer-dimer-tetramer assembly of FGF-2 at low HS-8 concentrations. The proposed self-association process for FGF-2 induced by HS-8 is illustrated in Fig. 4B.

The HS-8-induced monomer-dimer assembly of FGF-2 is consistent with the information currently known about FGF-heparin interactions. Calorimetric studies using low $M_r$ heparin ($M_r \sim$ 3000 or approximately 10 saccharide units) have shown that two FGF-2 molecules bind per heparin molecule (7). Cross-linking studies with a 16-saccharide heparin have also detected FGF-2 dimers and trimers (1, 11). In the study presented here, an active heparin fragment of minimal size (8 saccharide units) was used; yet, this short heparin fragment promotes the formation of FGF-2 dimers with a moderately high association constant. Since the site size for heparin binding is $\sim$4–5 saccharide units per FGF molecule (1, 7–9), this implies that in a complex with HS-8, FGF-2 molecules are in close proximity. There are three possibilities for the configuration of the FGF-2 molecules bound to HS-8: 1) the FGF-2 molecules are located in trans relative to the heparin; 2) the FGF-2 molecules are bound to neighboring sites on heparin but are not in contact with each other; 3) the FGF-2 molecules are bound to neighboring sites (in cis) on heparin and are in contact with each other. Venkataraman et al. (13) have argued that the third configuration is the most likely. Indeed, the crystal structures of
FGF-2 bound to heparin fragments or to nonsulfated heparan analogs (11, 12) suggest that HS-8 could bind across two adjacent FGF-2 monomers, stabilizing an FGF-2 “side by side” dimer cis to the heparin as illustrated in Fig. 4, B and C. Heparin has been reported by both NMR and x-ray diffraction studies to be a helical polysaccharide in solution with a 2-fold screw symmetry between successive disaccharide units (23). As a result, every fourth saccharide residue has approximately the same orientation with a translation of 16.5 Å along the heparin chain. An octasaccharide of heparin therefore consists of two side by side near-identical orientations of a tetrasaccharide, with an overall length of 33.0–33.4 Å. This corresponds closely to the width of the FGF-2 molecule (11, 13) and would be long enough to partially bind to the high-affinity heparin-binding sites (sites 1) of FGF-2. It is interesting to note that due to the 2-fold screw symmetry of heparin, the negatively charged binding surface available to the second dimer is different from that of the heparin-FGF-2 dimer. This configuration is consistent with the crystal structures of apo- and ligand-bound FGF-2, and is discussed in detail in Ref. 13.

Thus, the structural information currently available for heparin in solution and heparin:FGF-2 complexes are all consistent with the model of a heparin-induced side by side FGF-2 dimer cis to the heparin chain. We propose that this is the conformation of the HS-8-induced dimer that we report in this study. This putative stabilization of a side by side FGF-2 dimer by heparin is reminiscent of certain protein-DNA interactions, which show positive nearest-neighbor cooperativity upon binding of the protein to DNA.

A surprising result in the study presented here is that tetramers are observed at low HS-8 and salt concentrations. The formation of tetramer is likely to be caused by the binding of two additional FGF-2 monomers onto the original HS-8-induced dimer through their primary heparin-binding sites (sites 1 in Fig. 4B). Thus, the additional two FGF-2 monomers would form a secondary dimer that interacts with the first dimer through a head to head association, trans to the original dimer, as shown in Fig. 4B. The effect of salt on HS-8-induced association is informative; tetramerization appears to be abolished at salt concentrations greater than 50 mM, well below physiological levels (140 mM), while a monomer-dimer equilibrium exists at up to 500 mM NaCl. This indicates that the tetramerization process is primarily electrostatic in nature, and therefore is likely to involve the highly positively charged heparin-binding sites (sites 1) of FGF-2. It is interesting to note that due to the 2-fold screw symmetry of heparin, the negatively charged binding surface available to the second dimer is different from that bound by the original dimer, and based on our sedimentation results, appears to make weak electrostatic contacts with FGF-2. In contrast, the original dimer is much more stable to competition by salt, which is consistent with the side by side
dimer proposed by Venkataraman et al. (13), which is stabilized by a primarily uncharged protein-protein interface. The salt studies presented here show that the physiologically relevant association process induced by HS-8 is a monomer-dimer equilibrium, most likely with a side by side cis dimer conformation.

Another surprising result of this study is that SOS, like HS-8, induces a monomer-dimer equilibrium assembly of FGF-2; however, unlike HS-8, SOS is a biologically inactive compound. Unlike HS-8, SOS is not long enough to bind and bridge two sites 1 of a side by side FGF-2 dimer. Therefore, SOS must form a dimer by interacting simultaneously with two FGF-2 monomers by a divalent binding process. The binding site expected for negatively-charged SOS would be the primary heparin-binding site (site 1 in Fig. 4A), as it is a region of FGF-2 with very high positive electrostatic potential. This binding site corresponds to the observed binding site for SOS on FGF-1, a homologue of FGF-2 (15). A dimer formed by simultaneous binding of two FGF-2s to one SOS would thus be a head to head trans dimer where FGF-2 molecules are trans to the SOS ligand (Fig. 4A), with SOS presenting two anionic surfaces for binding. If SOS induces formation of a head to head trans dimer through electrostatic interaction with the positively-charged heparin-binding sites of two FGF-2s, then we would expect the trans dimerization to be sensitive to competition by salt. This is
supported by the observation that the SOS-induced dimer was only stable to 250 mM NaCl (see Fig. 3B). Unlike HS-8, no inhibition of association was seen even at high SOS concentrations (up to 952 μM). A study of SOS binding to FGF-2 (20) reported two binding sites with dissociation constants of 640 ± 130 μM (assuming two identical, independent sites, both located within the high-affinity heparin-binding site) and reported half-saturation (0.97 SOS bound per FGF-2 monomer) at 900 μM SOS. This is consistent with our results showing increasing dimerization up to 952 μM SOS, since inhibition of dimerization should only occur as the binding site is saturated.

The results presented here show that FGF-2 can dimerize in a heparin-dependent fashion, with dimerization dissociation constants in the low micromolar range. Micromolar protein concentrations are higher than typical levels for cytosolic soluble proteins; however, the physiologically relevant parameters in this system are the local concentrations of FGF-2 and heparin or heparan sulfate proteoglycan at the cell surface. The dimerization constants reported here have been determined using free FGF-2 and free heparin ligands capable of diffusion in three dimensions. In a physiological setting, however, FGF-2 is typically sequestered by HSPGs on the cell surface and in the extracellular matrix (21, 24). Therefore, the HSPG ligands are restricted to two-dimensional diffusion on the cell surface and the diffusion of the FGF-2 molecules is constrained by binding to the two-dimensionally restricted HSPGs (25). Schlessinger (26) showed that restriction of diffusion from three dimensions to a two-dimensional surface greatly increases the effective local concentration of a molecule. For example, 10^6 to 10^8 epidermal growth factor (EGF) receptors per cell is equivalent to an effective receptor concentration of 1–10 μM (27). Lemmon et al. (27) reported that the dimerization dissociation constant for the active EGF-EGF receptor complex is 3.3 μM, so the effective EGF receptor concentration is high enough for formation of the active complex (27). For comparison, there are typically 10^5–10^6 HSPG molecules per cell (28); therefore, the local concentrations of HSPG and sequestered FGF-2 are likely to reach levels sufficient for dimerization to occur.

It is also conceivable that both cis and trans dimer conformations are physiologically relevant. Heparan sulfate chains in HSPGs show distinct regions with different characteristic sequences and sulfation patterns along their length (29). Furthermore, activity of heparan sulfate is intimately linked to sulfation level; hypersulfation of HSPG can inhibit FGF-2 activity (30). It is therefore possible that hypersulfated regions of heparan sulfate more closely mimic SOS and could be involved in sequestering FGF-2 in a head to head inactive trans dimer. Alternatively, moderately sulfated regions of HSPGs would be expected to act similarly to HS-8, promoting the formation of active cis dimers. Pharmaceutical compounds that selectively induce either a trans or a cis FGF-2 dimer could be very useful, as they presumably would shift the conformation of FGF-2 between the putative inactive storage form and the mitogenically active cis dimer.

Several growth factor-responsive signaling pathways involve the binding of oligomeric growth factors to their cell-surface receptors. Examples of oligomeric growth factors for which ligand-receptor crystal structures are known include interferon-γ (31), a homodimer, and tumor necrosis factor-β (32), a homotrimer. Each ligand molecule presents an identical binding surface to a receptor subunit, thereby recruiting a receptor dimer or trimer. Other examples of homodimeric protein ligands that bind multiple receptors are stem cell factor binding to the Kit receptor (33), nerve growth factor and other neurotrophins binding to p75 and trk family receptors (34), and glial cell line-derived neurotrophin (a disulfide-linked dimer) which binds two glial cell line-derived neurotrophic factor receptor-α molecules and 2 Ret receptors (35). Other protein ligands are monomeric in solution, yet form 2:2 complexes with receptor, such as EGF (27) and granulocyte-colony stimulating factor (36).

A recent study by Spivak-Kroizman et al. (9) of FGF-1 and the extracellular domain of FGFR2 showed that heparin induces dimerization of two 1:1 FGF-1:FGFR2 complexes and that in the presence of heparin, 2:2 complexes of FGF-1:FGFR2 formed on the cell surface. Contradictory results, however, have been reported for FGF-2. Pantoliano et al. (16) used isotermal titration calorimetry and sedimentation equilibrium studies to analyze the interactions of FGF-2 with a 2-immunoglobulin domain form of FGFR1. They found that FGF-2 formed a 1:2 complex with the receptor in the presence of saturating amounts of heparin. Our model for heparin-induced FGF-2 dimerization appears to be consistent with the findings of Spivak-Kroizman et al. (9). However, in the absence of information about the stoichiometry of FGF-2:FGFR1 complexes on the cell surface, it is not clear which mechanism occurs in vivo. FGF receptors show different expression patterns, selectivity for FGF ligands, and splice variations (37); therefore, different combinations of receptor and ligand may employ alternate activation mechanisms.

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