Kit Receptor Dimerization Is Driven by Bivalent Binding of Stem Cell Factor*

(Received for publication, October 7, 1996, and in revised form, December 23, 1996)

Mark A. Lemmon‡, Dalia Pinchasi, Min Zhou§, Irit Lax, and Joseph Schlessinger¶

From the Department of Pharmacology, New York University Medical Center, New York, New York 10016

Most growth factors and cytokines activate their receptors by inducing dimerization upon binding. We have studied binding of the dimeric cytokine stem cell factor (SCF) to the extracellular domain of its receptor Kit, which is a receptor tyrosine kinase similar to the receptors for platelet-derived growth factor and colony-stimulating factor-1. Crystallographic studies show that one SCF dimer binds simultaneously to two molecules of the Kit extracellular domain. Gel filtration and other methods show that this results in Kit dimerization. It has been proposed that SCF-induced Kit dimerization proceeds via a conformational change that exposes a key receptor dimerization site in the fourth of the five immunoglobulin (Ig)-like domains in Kit. We show that a form of Kit containing just the first three Ig domains (Kit-123) binds to SCF with precisely the same thermodynamic parameters as does Kit-12345. Analytical ultracentrifugation, light scattering, and gel filtration show that Kit-123 dimerizes upon SCF binding in a manner indistinguishable from that seen with Kit-12345. These data argue that the fourth Ig-like domain of Kit is not required for SCF-induced receptor dimerization and provide additional support for a model in which bivalent binding of the SCF dimer provides the driving force for Kit dimerization.

‡Marion Abbe Fellow of the Damon Runyon-Walter Winchell Cancer Research Foundation (DRG-1243). Present address: Dept. of Pharmacology, New York University Medical Center, New York, New York 10016.
§Present address: Dept. of Molecular Biology, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543.
¶To whom correspondence should be addressed: Dept. of Pharmacology, New York University Medical Center, 550 First Ave., New York, NY 10016. Tel.: 212-263-7111; Fax: 212-263-7133.

*This work was supported in part by a grant from Sugen (to J. S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Ligand-induced receptor dimerization provides the mechanism for transmembrane signaling by many receptors with a single transmembrane domain (1–3). Crystallographic and other biophysical studies have shown that this results from bivalent binding of the cognate ligand to the receptors for human growth hormone (hGH) (4, 5) and interferon-γ (IFN-γ) (6). hGH is a monomeric cytokine that has two receptor binding sites. Each site binds to a separate receptor molecule, forming a 1:2 (ligand:receptor) complex (4), with a 1:1 monomeric receptor complex occurring as an intermediate (4, 7). An x-ray crystal structure of the hGH-hGH-R complex shows that, in addition to ligand-mediated interactions, receptor-receptor interactions also contribute to stabilization of the receptor dimer (5). IFN-γ is a dimeric cytokine, and one IFN-γ dimer binds simultaneously to two molecules of the α-chain of its receptor. An x-ray crystal structure of the complex between IFN-γ and the extracellular domain of the IFN-γ receptor α-chain (IFN-γRα) shows that the dimer is stabilized solely through ligand-mediated interactions (6). In another case of ligand-induced receptor oligomerization for which high resolution structural information is available, that of the tumor necrosis factor receptor, the ligand (tumor necrosis factor-β) is a trimer that binds simultaneously to three receptor molecules. Each receptor extracellular domain contacts two promoters of the ligand trimer, and no direct receptor-receptor contact is seen in the trimeric complex (8).

Although no detailed structural information exists for activated receptor tyrosine kinases in complex with their ligands, many reports indicate that bivalent binding of growth factors is key for inducing receptor dimerization (reviewed in Refs. 2 and 3). For example, platelet-derived growth factor (PDGF) is a disulfide-linked dimer that binds to two PDGF-receptor molecules (9, 10). The ligands for the Trk receptors also form dimers to which two receptor molecules can bind, resulting in receptor dimerization (11). Finally, acidic fibroblast growth factor (aFGF), although itself monomeric and incapable of inducing dimerization of its receptor, forms a multivalent complex with heparan sulfate proteoglycans that can in turn bind to two or more receptors and thus stabilize active FGF receptor dimers (12).

In the process of our investigations of the mechanisms of growth factor-induced dimerization of receptor tyrosine kinases, we have studied the binding of stem cell factor (SCF) to the extracellular domain of its receptor, Kit, a class III receptor tyrosine kinase with similarity to the receptors for colony stimulating factor-1 (CSF-1) and PDGF (1). Kit contains a ligand-binding extracellular domain of 520 amino acids, a single transmembrane domain, and an intracellular tyrosine kinase domain. Like CSF-1 and PDGF, SCF is dimeric in solution (13), and has been shown to induce the dimerization of both the full-length Kit receptor (with resulting activation) and the isolated Kit extracellular domain (14, 15). As with the PDGF and CSF-1 receptors, the Kit extracellular domain consists of five immunoglobulin (Ig)-like domains. The first three of these domains are primarily responsible for SCF binding to Kit, as shown in interspecies domain swapping experiments (16) and studies of monoclonal antibodies that inhibit SCF binding (17). The remaining two Ig-like domains have been proposed to play a role in stabilizing the SCF-induced Kit dimer (18). A mechanism for Kit activation has been proposed in which monovalent binding of SCF to a single molecule of Kit induces a conformational change in the receptor that in turn exposes a receptor dimerization site (15). It was further reported (18)
that the fourth Ig-like domain of Kit represents the key ligand-induced receptor dimerization site. Monoclonal antibodies against this domain inhibited SCF-induced Kit activation; deletion of the fourth domain abolished Kit function (and reduced SCF binding); and a form of the Kit extracellular domain containing only the first three Ig-like domains did not form cross-linked dimers upon SCF treatment.

In most studies, growth factor receptor dimerization has been analyzed in intact cells or in detergent-solubilized preparations, using approaches such as covalent cross-linking and antibody inhibition that do not permit a quantitative analysis. We have generated large quantities of the extracellular domains of several growth factor receptors for structural studies, but have so far been unable to obtain sufficiently good crystals for x-ray crystallographic analysis. In the absence of a detailed structural view of growth factor-induced receptor dimerization, we have used several biophysical methods to explore this process. In this report, we describe studies of SCF binding to the isolated Kit extracellular domain, using titration calorimetry, analytical ultracentrifugation, and size-exclusion chromatography. We show that a single bivalent SCF dimer binds simultaneously to two molecules of Kit, and thus induces its dimerization. By contrast with the findings of Blechman et al. (18), we find that a version of Kit containing just the first three Ig-like domains (Kit-123) binds to SCF and dimerizes in an manner identical to that seen with the complete extracellular domain (Kit-12345). Our data provide further evidence for the importance of SCF bivalence in Kit dimerization, through an explicit measurement of binding stoichiometry, and refute the proposal that a significant ligand-induced Kit dimerization site is present in the fourth Ig-like domain of Kit.

**EXPERIMENTAL PROCEDURES**

*Production and Purification of Proteins—* DNA encoding amino acids 1–141 of human SCF was subcloned into pET11a (Novagen) and expressed in *Escherichia coli* MT7. After cell lysis, SCF in the insoluble pellet fraction was solubilized with 8 M urea, from which it was refolded by dilution in the presence of 1 M reduced glutathione essentially as described by Saltzman et al. (19). The refolded material was purified on an anion-exchange chromatography column in 10 mM Tris-HCl, pH 8.0, using a FastFlow Q column (Pharmacia Biotech Inc.) followed by gel filtration on a Superdex 75 fast protein liquid chromatography (Pharmacia) column in 50 mM Tris-HCl, pH 8.0, 250 mM NaCl. The resulting material was better than 95% pure as assessed by Coomassie staining of an overloaded SDS-polyacrylamide gel.

For expression of Kit-12345 (residues 1–590) and Kit-123 (residues 1–319), stop codons were introduced by site-directed mutagenesis at the desired positions in the cDNA encoding human c-Kit. Mutated Kit cDNA fragments were then subcloned between the BamHI and HindIII sites of the BlueBac III baculovirus transfer vector (Invitrogen), and plasmid was cotransfected with BaculoGold DNA (Pharmingen) into *Sf9* insect cells. Recombinant virus stocks were amplified using High Five cells (In vitrogen) to yield a high titer virus stock. Protein was secreted into medium by *Sf9* cells infected with this virus stock for 48–72 h. Kit extracellular domains were then purified from conditioned medium using immunoadfinity chromatography (for Kit-12345) or a column containing SCF on CnBr-activated beads (for Kit-123). Kit protein was eluted using ActiSep, dialyzed exhaustively against 20 mM HEPES, pH 7.5, and further purified by anion-exchange chromatography on a Mono-Q column followed by gel filtration using a Superose-12 fast protein liquid chromatography column (Pharmacia). The final protein product was seen to be greater than 95% pure in each case by Coomassie staining of overloaded SDS-polyacrylamide gels.

Protein concentrations were measured spectrophotometrically, using calculated molar extinction coefficients (9970 M⁻¹ cm⁻¹ for SCF, 36800 M⁻¹ cm⁻¹ for Kit-123 and 68250 M⁻¹ cm⁻¹ for Kit-12345). Quantitative amino acid analysis revealed the several samples with known absorbance showed these values to be correct to within 10%.

**Isothermal Titration Calorimetry—** The OMEGA instrument from MicroCal (20) was used, to which access was kindly provided by Professor Julian Sturtevant (Department of Chemistry, Yale University). Titrations were performed at 25 °C in 50 mM HEPES, 150 mM NaCl, pH 7.5, against which both SCF and Kit protein had been exhaustively dialyzed prior to titration. Controls for heats of dilution were performed as described previously (21) and found to remain constant throughout each titration. Values for the heat of dilution were subtracted from the heats measured in each titration prior to fitting the data. The value for [Kit]₀ ([c value] in each experiment was between 20 and 60. Experimental titration curves were fit using ORIGIN software (MicroCal) as described elsewhere (20), assuming a single class of binding site as indicated.

**Dynamic Light Scattering, Size-exclusion Chromatography, and Analytical Ultracentrifugation—** All experiments were performed at 25 °C in 50 mM HEPES, 150 mM NaCl, pH 7.5. Dynamic light scattering experiments employed the Protein Solutions instrument, to which access was kindly provided by Prof. Rashmi Hegde (Skirball Institute).

Size-exclusion chromatography was performed using a Bio-Sil SEC 250 column (50 × 7.8 mm) with a Bio-Sil guard column (80 × 7.8 mm) (Bio-Rad) attached to an LKB high performance liquid chromatography system, running at 0.5 ml/min.

Sedimentation equilibrium experiments employed the XL-A analytical ultracentrifuge (Beckman) in the laboratory of Professor Steven K. Burley (Rockefeller University), kindly aided by Elaine Halay. Concentration distributions were monitored at 230, 260, or 280 nm. Experiments were performed at 25 °C using six-channel cells at three different speeds (8500, 10200, and 10500 rpm) with identical results. Kit-123 and SCF were analyzed separately at the concentrations shown in Table II. For analysis of the SCF-Kit-123 complex, an equimolar mixture of the two components was run at the Kit-123 concentrations noted. Samples were equilibrated at each speed for greater than 18 h. Protein partial specific volumes were estimated from their amino acid compositions (0.74 ml/g for SCF, and 0.715 ml/g for Kit-123, assuming approximately 15% (w/v) oligosaccharide), and solvent density was taken as 1.003 g/ml. Data were fit using the Optima XL-A data analysis software (Beckman/MicroCal). In each case, a fit to a single ideal species gave the most randomly scattered residuals.

**RESULTS AND DISCUSSION**

Using a baculovirus expression system we have generated and purified milligram quantities of the complete extracellular domain of human Kit (Kit-12345), as well as a form from which Ig-like domains 4 and 5 have been removed (Kit-123) (see “Experimental Procedures”). Human SCF (1–141) was expressed in E. coli, using a T7 expression system, and was refolded and purified using a procedure described by Langley et al. (19). Binding of SCF to the two different forms of the Kit extracellular domain was studied using isothermal titration calorimetry (ITC). SCF-induced dimerization of Kit was also monitored, using size-exclusion chromatography (SEC), analytical ultracentrifugation, chemical cross-linking and dynamic light scattering. We find that both Kit-12345 and Kit-123 behave identically with respect to both SCF binding and their resulting dimerization. A single bivalent SCF dimer binds simultaneously to two molecules of Kit-12345 or Kit-123, resulting in dimerization of the receptor extracellular domain.

**Oligomeric State of SCF and Kit Extracellular Domain—** Before proceeding with an analysis of ligand-induced receptor dimerization, it is important to determine the oligomeric state of the ligand and the receptor extracellular domains under the experimental conditions employed. Using SEC and analytical ultracentrifugation, we found that both unliganded Kit-12345 and unliganded Kit-123 behave as monomers of their expected molecular weight (plus mass of carbohydrate), and that free SCF (1–141) behaves as a dimer of 26 kDa, as expected (see Table II and Fig. 2).

**Binding of SCF to Kit-12345**—ITC was used to monitor SCF binding to Kit-12345. The data obtained upon titration of SCF into a solution of Kit-12345 (present in the calorimeter cell) demonstrate the existence of a single class of thermo-dynamically equivalent binding sites (Fig. 1, upper panel). Since heat is released throughout the titration, the binding reaction is exothermic (ΔH = −8.7 kcal mol⁻¹) and predominantly enthalpy-driven at 25 °C. Fitting of several titrations such as that in Fig. 1 gave an apparent binding constant (K₅) for SCF binding to Kit-12345 of 1.8 × 10⁶ M⁻¹ (K_D(app) = 55 nM) (Table I), and a
stoichiometry of 1:1 (SCF:Kit-12345). Since SCF is a dimer under the conditions of this experiment, this measured stoichiometry requires that a single dimer of SCF binds simultaneously to two molecules of Kit-12345, thus inducing Kit-12345 dimerization. Identical titrations were obtained when the experiment was reversed, with Kit-12345 being titrated into a solution of SCF (not shown). The apparent $K_D$ reported here (55 nM) is higher (by around 20-fold) than values reported for SCF binding to Kit on the surface of cells ($K_D \approx 2$ nM) (15, 16) or to the immobilized Kit extracellular domain (14). Similar differences occur with other receptors, for example those for aFGF (12), EGF (22), and hGH (7) when ligand binding to the extracellular domain (free in solution) is compared with binding to the intact receptor. There are several likely explanations for this difference. Studies of SCF binding to intact Kit have required the interpretation of nonlinear Scatchard plots (15–18), which is plagued with quantitative difficulties (23, 24). The fact that the ligand in this case (SCF) is also bivalent creates further difficulties in interpretation (25) that have not been adequately addressed. In addition to these considerations, the apparent affinity will be elevated by an avidity effect that arises from the high local concentration of receptor when restricted to the cell membrane or immobilized artificially. Furthermore, each of the binding events is coupled to receptor dimerization, and it can readily be shown that dimerization of membrane-localized receptors is greatly favored over their association when tumbling freely in solution (26). Therefore, it is expected that the apparent $K_D$ for ligand interacting with the free receptor extracellular domain will be higher than that for binding to the membrane-localized receptor. In studies similar to those reported here, Philo et al. (27) determined a $K_D$ for SCF/Kit-12345 binding, when both components were free in solution, that agrees closely with our value.

![Graph](image)

**Fig. 1.** Titration calorimetry of SCF binding to Kit-12345 (upper panel) and Kit-123 (lower panel) showing that the stoichiometry of which is indistinguishable from those obtained with Kit-12345 (Fig. 1, lower panel). $K_D^{(app)}$ for this interaction was 49.2 nM, $\Delta H$ was $-10.2$ kcal mol$^{-1}$, and the stoichiometry was close to 1:1 (SCF:Kit-123). These thermodynamic parameters are indistinguishable from those obtained with Kit-12345 (Table I). Therefore, removal of Ig-like domains 4 and 5 from the Kit extracellular domain has no influence upon the thermodynamics of SCF binding. This result is in agreement with the reports that Ig-like domains 1–3 are of primary importance in ligand binding to Kit (16, 17) and shows that domains 4 and 5 do not contribute at all. However, since SCF binding is coupled to the dimerization of both Kit-12345 and -123 (see below), our results are inconsistent with the hypothesis (18) that Ig-like domains 4 and/or 5 contribute in a significant way to SCF-induced Kit dimerization.

**Binding of SCF to Kit-123**—Identical ITC studies were repeated for Kit-123. Titrations of SCF into Kit-123, and of Kit-123 into a solution of SCF, gave binding isotherms that were indistinguishable from those obtained with Kit-12345 (Fig. 1, lower panel). $K_D^{(app)}$ for this interaction was 49.2 nM, $\Delta H$ was $-10.2$ kcal mol$^{-1}$, and the stoichiometry was close to 1:1 (SCF:Kit-123). These thermodynamic parameters are indistinguishable from those obtained with Kit-12345 (Table I). Therefore, removal of Ig-like domains 4 and 5 from the Kit extracellular domain has no influence upon the thermodynamics of SCF binding. This result is in agreement with the reports that Ig-like domains 1–3 are of primary importance in ligand binding to Kit (16, 17) and shows that domains 4 and 5 do not contribute at all. However, since SCF binding is coupled to the dimerization of both Kit-12345 and -123 (see below), our results are inconsistent with the hypothesis (18) that Ig-like domains 4 and/or 5 contribute in a significant way to SCF-induced Kit dimerization.

**Dimerization of Kit-12345 upon SCF Binding**—To further test our interpretation of the ITC results for SCF binding to Kit-12345, we used chemical cross-linking and SEC to monitor dimerization of the receptor extracellular domain upon ligand binding. In agreement with previous reports (14, 18), we could detect significant SCF-dependent formation of covalently linked Kit-12345 dimers in chemical cross-linking experiments (data not shown). SCF-induced dimerization was also apparent in SEC studies (Fig. 2). Kit-12345 loaded alone eluted at a

**Table I**

| Kit protein | $K_D^{(app)}$ | $K_D^{(app)}$ | Stoichiometry | $\Delta H$ | $\Delta G$ |
|-------------|--------------|--------------|---------------|-----------|----------|
| Kit-12345   | 2.02 ± 0.57  | 49.5         | 1.01 ± 0.02   | -8.02 ± 0.12 | -9.98    |
| Kit-12345   | 1.98 ± 0.40  | 51.0         | 1.04 ± 0.01   | -7.65 ± 0.10 | -9.83    |
| Kit-12345   | 1.56 ± 0.32  | 64.1         | 1.06 ± 0.02   | -10.5 ± 0.3 | -9.82    |
| Mean 12345  | 1.85 ± 0.25  | 54.9 ± 8.0   | 1.04 ± 0.02   | -8.72 ± 1.55 | -9.88 ± 0.09 |
| Kit-123     | 2.17 ± 0.36  | 46.1         | 1.11 ± 0.01   | -9.90 ± 0.14 | -10.01   |
| Kit-123     | 1.91 ± 0.41  | 52.3         | 1.12 ± 0.01   | -10.4 ± 0.2 | -9.94    |
| Mean 123    | 2.04 ± 0.18  | 49.2 ± 4.4   | 1.11 ± 0.01   | -10.2 ± 0.4 | -9.98 ± 0.05 |

* In each case titrations of SCF into Kit gave results identical to those for titrations of Kit into SCF.
* Curve fitting for apparent binding constants and stoichiometry assumes a single class of binding sites: $K_D^{(app)} = 1/K_D^{(app)}$.
* Stoichiometry represents SCF monomer:Kit monomer ratio.
position corresponding well to its expected monomeric molecular weight (80 kDa). Addition of SCF at the molar ratios noted in Fig. 2 resulted in the appearance of a new peak, eluting at the position expected for a dimeric (SCF)₂(Kit-12345)₂ complex (~190 kDa) upon addition of one SCF dimer per two Kit-12345 molecules. Ligand added in excess of this ratio runs as free dimeric SCF. The position and amplitude of the (SCF)₂(Kit-12345)₂ peak was unaffected at SCF excesses up to 100-fold (not shown). According to the positions of the molecular mass standards shown at the bottom of the figure, the complex between SCF and Kit-12345 must contain an SCF dimer and two molecules (a dimer) of Kit-12345.

SCF-induced Dimerization of Kit-123—Identical experiments employing SEC were also performed with the truncated form of Kit containing only the first three immunoglobulin domains (Kit-123). The results obtained using Kit-123 were identical to those obtained with Kit-12345 (Fig. 3), showing that one dimer of SCF binds to Kit-123 and induces its dimerization. As with the complete extracellular domain, no evidence for the formation of a complex containing a single Kit-123 molecule bound to an SCF dimer was obtained. These results were corroborated in studies employing dynamic light scattering, which gave an approximate molecular mass of 62 kDa for Kit-123 alone, and 114 kDa for the SCF-Kit-123 complex; again suggesting SCF-induced Kit-123 dimerization. However, in agreement with the finding of Blechman et al. (18), we could not detect SCF-induced Kit-123 dimerization by chemical...
cross-linking analysis. We therefore sought additional evidence for SCF-induced Kit-123 dimerization, performing sedimentation equilibrium experiments with an analytical ultracentrifuge. As reported in Table II, SCF (1–141) itself sedimented in the ultracentrifuge as a single species that represents a dimer of 28.2 ± 1.2 kDa, while unliganded Kit-123 sedimented as a monomer of 29.2 ± 1.5 kDa (slightly below the expected molecular mass). When a series of concentrations of a 1:1 mixture of SCF and Kit-123 was analyzed, the residuals for the fits to the data were most clearly random, for each of the three rotor speeds employed, when a single ideal species was assumed (Fig. 4). This single species sedimented with a molecular mass of 79.3 ± 1.5 kDa, which can be accommodated (given the known stoichiometry) only by the formation of a complex containing one SCF dimer and two molecules of Kit-123 (Table II). Thus, with the exception of chemical cross-linking studies, each of the physical techniques employed here shows that Kit-123, like Kit-12345, dimerizes quantitatively upon binding of one SCF dimer to two molecules of Kit-123.

The Fourth Immunoglobulin-like Domain of Kit Is Not Required for Dimerization—Since dimerization of the Kit extracellular domain is coupled to SCF binding (Figs. 2 and 3), and the thermodynamics of SCF binding are unaffected by the removal of Ig domains 4 and 5 (Table I), the fourth Ig-like domain of Kit cannot represent a thermodynamically significant receptor dimerization site. The model of Blechman et al. (18) proposes that one SCF dimer binds to one Kit molecule, and induces a conformational change that exposes a key receptor dimerization site in the fourth Ig-like domain. Kit dimerization is suggested not to occur when this site is blocked or removed. According to this model, Kit-123 should not dimerize upon SCF binding. Furthermore, its SCF binding should be weaker than that of Kit-12345 since bivalent ligand binding to the latter is coupled to receptor dimerization. Any loss of receptor-receptor interactions involving the putative dimerization site in domain 4 should therefore be clearly reflected in a comparison of the thermodynamics of SCF binding to Kit-12345 and Kit-123. We could detect no difference in SCF binding to the two forms of Kit, and our data show that Kit-123 dimerizes upon SCF binding in a manner indistinguishable from that seen for Kit-12345. The fact that we were unable to detect the binding of one SCF dimer to a single Kit-123 or Kit-12345 monomer does argue that receptor-receptor interactions may participate in Kit dimerization to some extent. However, these interactions are not significantly impaired upon removal of both Ig-like domains 4 and 5.

The role of the fourth Ig-like domain in Kit dimerization was primarily suggested by the ability of a monoclonal antibody raised against this domain to inhibit SCF-induced Kit activation and to prevent cross-linking of SCF to full-length Kit dimers (18). Furthermore, it was found that full-length Kit from which the fourth Ig-like domain had been deleted (Kit-Δ4) could not be activated and did not form SCF-induced dimers in cross-linking experiments. Finally, Kit-123 could not be shown to dimerize upon SCF binding in chemical cross-linking experiments, while Kit-12345 could (18). The biophysical analyses presented here, using four different methods, show clearly that Kit-123 dimerizes upon SCF binding, with parameters that are indistinguishable from those seen with Kit-12345. We therefore suggest the possibility that the monoclonal antibody against the fourth Ig-like domain employed by Blechman et al. (18) could exert its influence through steric effects upon receptor dimerization. The inability to cross-link Kit-123 dimers in the presence of SCF, which we also experienced, could simply reflect a difference in the availability of reactive groups in the two forms of the Kit extracellular domain. Clearly, from our SEC, dynamic light scattering, and centrifugation studies, SCF does induce Kit-123 dimerization. The results reported with the Kit-Δ4 mutant that would not dimerize or signal (18) are more difficult to explain, but could reflect deleterious confor-

### Table II

| Protein | Concentration | Molecular massa | Oligomeric state | Kit-123:SCF Ratio |
|---------|---------------|-----------------|-----------------|------------------|
| SCF     | 54.2          | 27.9 ± 1.4      | Dimerb          |                  |
| SCF     | 10.0          | 28.3 ± 0.7      | Dimerb          |                  |
| Mean    | 28.2 ± 1.2    | Dimerb          |                 |                  |
| Kit-123 | 18.2          | 30.2 ± 0.8      |                 |                  |
| Kit-123 | 2.2           | 28.2 ± 1.4      |                 |                  |
| Mean Kit-123 | 29.2 ± 1.5 | Monomerb          |                  |                  |
| SCF + Kit-123 | 8.7      | 77.9 ± 2.0      | 1.70:2          |                  |
| SCF + Kit-123 | 8.0      | 80.5 ± 1.0      | 1.79:2          |                  |
| SCF + Kit-123 | 6.4      | 80.7 ± 0.9      | 1.80:2          |                  |
| SCF + Kit-123 | 1.6      | 78.2 ± 0.6      | 1.71:2          |                  |
| Mean complex | 79.3 ± 1.5 | 2 SCF: 2 Kit-123 | 1.75:2          |                  |

a Molecular masses are based upon a fit to a single ideal species using the Optima XL-A data analysis software. In each case, the residuals for the fit were randomly distributed (see Fig. 5 for an example). Identical results were obtained at each of the three rotor speeds used.

b Molecular masses for both SCF and Kit-123 are smaller than suggested by their amino acid sequences (by approximately 10–15%). This may reflect some C-terminal proteolytic degradation of the species or may result from the presence of small quantities of low molecular mass impurities in the samples. Fits of the data to two independent species could not adequately account for the discrepancy.

![Fig. 4. Sedimentation equilibrium centrifugation of a 1:1 mixture of SCF and Kit-123. The data (open circles) are fitted to a curve describing the concentration distribution of a single ideal species using the Optima XL-A data analysis software. A plot of the residuals for this fit (upper panel) shows that they are distributed reasonably randomly, indicative of a good fit. In this particular case, the molecular mass was 80.5 ± 1.0 kDa, with χ² = 0.00029. This would correspond to a species containing one SCF dimer and 1.79 molecules of Kit-123, or a mixture of components with 90% (by mass) in the form of an 86.8-kDa (SCF)₂/(Kit-123), dimer and 10% as free Kit-123 or SCF.](image-url)
the Kit extracellular domain cooperates with weak receptor-receptor interactions (that are not abolished upon removal of the fourth and fifth Ig-like domains) to drive dimer formation. The model is very similar to those for IFN-γ binding to the α-chain of its receptor (6), and binding of an aFGF-heparin complex to the FGF receptor (12, 28). In each of these cases, a multimeric ligand (HS PG-induced in the case of aFGF) stabilizes receptor oligomers by virtue of its multivalent receptor binding (12, 30). It is also very similar to the model for hGH-induced dimerization of hGHR, except that in this case the bivalent ligand is a monomer. While receptor-receptor contacts are clearly observed in the crystal structure of the hGH-induced hGHR dimer (5, 29), no receptor-receptor contacts are evident in the crystal structure of IFN-γ bound to two extracellular domains of IFN-γRa (6). We have also not observed thermodynamically significant interactions between the two protomers of the aFGF-induced dimer of the FGF receptor extracellular domain (12).

Studies of SCF binding to CHO-cell derived Kit-12345, employing analytical ultracentrifugation, SEC, and ITC were recently reported by Philo et al. (27), with results very similar to those presented here, showing that one SCF dimer binds to two molecules of Kit-12345. The thermodynamic parameters described for CHO-cell derived binding to E. coli-derived SCF (1–165) are in accord with our measurements for E. coli-derived SCF (1–141) binding to baculovirus-generated Kit-12345. In agreement with our finding, Philo et al. (27) did not observe disruption by excess SCF of the (SCF)2/(Kit-12345)2 dimeric complex in SEC studies. However, detailed model-fitting of analytical ultracentrifugation data suggested that the (SCF)2/Kit-12345 monomeric complex does occur to a significant extent. It was thus concluded that there is little cooperativity in Kit-12345 binding to the SCF dimer, indicating that inter-receptor interactions are likely to be weak.

According to the conclusions of Blechman et al. (18), if the fourth Ig-like domain represents a key ligand-induced Kit dimerization site, significant cooperativity in Kit-12345 binding to dimeric SCF would be observed, and Kit-123 would not dimerize. Our results and those of Philo et al. (27) make a strong case against this assertion. Possible origins for this discrepancy are outlined above. Lev et al. (15) and Blechman et al. (18) have also argued against the importance of SCF bivalency in inducing Kit dimerization, largely on the basis of the absence of a bell-shaped dose-response curve for SCF (although this is seen in some experiments). Our inability to detect monomeric SCF:Kit complexes represents an analogous observation. However, as pointed out by Wells (30), a bell-shaped dose-response curve (interpreted as an indication of dimer disruption) may be difficult to detect in cases where bivalent ligand binding drives dimerization. With the cooperation of bivalent ligand/receptor interactions and receptor/receptor interactions, extremely high ligand concentrations may be required to disrupt the dimer. For example, the concentration of hGH required to disrupt an hGH-induced dimer of membrane-bound hGHR dimer is some 10,000-fold higher than the hGH concentration required for a maximal cellular response. Such high excesses have not been studied for SCF. As with hGH/hGHR, therefore, we argue that the subunits of an activated Kit dimer are cooperatively linked both by a bivalent cross-linking ligand and receptor-receptor interactions.

Acknowledgments—We thank Julian Sturtevant (Yale University) for kindly allowing access to the MicroCal OMEGA titration calorimeter (supported by National Institutes of Health Grant GM04725); Stephen Burley and Elaine Halay (Rockefeller University) for access to and assistance with the Beckman XL-A analytical ultracentrifuge; and Rashmi Hegde for access to the dynamic light scattering instrument. We are also grateful to Sima Lev and James Rice (Sugen, Inc.) for their...
help with SCF production, and Kathryn Ferguson, James A. Wells, and Donald Engelman for their critical reading of the manuscript.

REFERENCES
1. Ullrich, A., and Schlessinger, J. (1990) Cell 61, 203–212
2. Lemmon, M. A., and Schlessinger, J. (1994) Trends Biochem. Sci. 19, 459–463
3. Heldin, C.-H. (1995) Cell 80, 213–223
4. Cunningham, B. C., Ullsch, M., De Vos, A. M., Mulkerrin, M. G., Clauser, K. R., and Wells, J. A. (1991) Science 254, 821–825
5. De Vos, A. M., Ullsch, M., and Kossiakoff, A. A. (1992) Science 255, 306–312
6. Walter, M., Windsor, W. T., Nagabhushan, T. L., Lundell, D. J., Lunn, C. A., Zasrodny, P. J., and Narula, S. R. (1995) Nature 376, 230–235
7. Fuh, G., Cunningham, B. C., Fukunaga, R., Nagata, S., Goeddel, D. V., and Wells, J. A. (1992) Science 256, 1677–1680
8. Banner, D. W., D’Arcy, A., Janes, W., Gentz, R., Schoenfeld, H.-J., Broger, C., Loetscher, H., and Lesslauer, W. (1993) Cell 73, 431–445
9. Heldin, C.-H., Ernlund, A., Rorsman, C., and Rönstrand, L. (1989) J. Biol. Chem. 264, 8905–8912
10. Fretto, L. J., Snape, A. J., Tomlinson, J. E., Seroogy, J. J., Wolf, D. L., LaRochelle, W. J., and Giese, N. A. (1993) J. Biol. Chem. 268, 3625–3631
11. Philo, J., Talvenheim, J., Wen, J., Rosenfeld, R., Welcher, A., and Arakawa, T. (1994) J. Biol. Chem. 269, 27849–27856
12. Spivak-Kroizman, T., Lemmon, M. A., Kikkic, I., Ladbury, J. E., Pinchasi, D., Huang, J., Jaye, M., Crumley, G., Schlessinger, J., and Lax, I. (1994) Cell 79, 1015–1024
13. Arakawa, T., Yphantis, D. A., Lary, J. W., Narhi, L. O., Lu, H. S., Prestrelski, S. J., Clogston, C. L., Zeebo, K. M., Mendiaz, E. A., Wyppych, J., and Langley, K. E. (1991) J. Biol. Chem. 266, 18942–18948
14. Lev, S., Yarden, Y., and Givol, D. (1992) J. Biol. Chem. 267, 10866–10873
15. Lev, S., Yarden, Y., and Givol, D. (1992) J. Biol. Chem. 267, 15970–15977
16. Lev, S., Blechman, J., Nishikawa, S.-I., Givol, D., and Yarden, Y. (1993) Mol. Cell. Biol. 13, 2224–2234
17. Blechman, J. M., Lev, S., Brizi, M. F., Leitner, O., Pegoraro, L., Givol, D., and Yarden, Y. (1993) J. Biol. Chem. 268, 4399–4406
18. Blechman, J. M., Lev, S., Barg, J., Eisenstein, M., Vaks, B., Vogel, Z., Givol, D., and Yarden, Y. (1995) Cell 80, 103–113
19. Langley, K. E., Wyppych, J., Mendiaz, E. A., Clogston, C. L., Parker, V. P., Farrar, D. H., Brothers, M. O., Stogal, V. N., Leslie, I., Birkett, N. C., Smith, K. A., Balterman, R. F., Lyons, D. E., Hogan, J. M., Crandall, C., Boone, T. C., Pope, J. A., Karkare, S. B., Zeebo, K. M., Sachdev, R. K., and Lu, H. S. (1992) Arch. Biochem. Biophys. 295, 21–28
20. Wiseman, T., Willistin, S., Brandts, J. F., and Lin, L.-N. (1989) Anal. Biochem. 179, 131–137
21. Lemmon, M. A., and Ladbury, J. E. (1994) Biochemistry 33, 5070–5076
22. Lemmon, M. A., Bu, Z., Ladbury, J. E., Zhou, M., Pinchasi, D., Lax, I., Englander, D. M., and Schlessinger, J. (1997) EMBO J. 16, 281–294
23. Zierler, K. (1989) Trends Biochem. Sci. 14, 314–317
24. Klotz, I. (1982) Science 217, 1247–1249
25. Winzor, D. J., and Sawyer, W. H. (1995) Quantitative Characterization of Ligand Binding, pp. 104–107, Wiley-Liss, New York
26. Grabberger, B., Minton, A. P., DeLisi, C., and Metzger, H. (1986) Proc. Natl. Acad. Sci. U.S.A. 83, 6258–6262
27. Philo, J. S., Wen, J., Wyppych, J., Schwartz, M. G., Mendiaz, E. A., and Langley, K. E. (1996) J. Biol. Chem. 271, 6685–6692
28. Schlessinger, J., Lax, I., and Lemmon, M. A. (1995) Cell 83, 357–360
29. Kossiakoff, A. A., Somers, W., Ullsch, M., Andow, K., Muller, Y. A., and De Vos, A. M. (1994) Protein Sci. 3, 1697–1705
30. Wells, J. A. (1996) Proc. Natl. Acad. Sci. U.S.A. 93, 1–6