Human Stem Cell Factor Dimer Forms a Complex with Two Molecules of the Extracellular Domain of Its Receptor, Kit*

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Stem cell factor (SCF) is a cytokine that is active toward hematopoietic progenitor cells and other cell types, including germ cells, melanocytes, and mast cells, which express its receptor, the tyrosine kinase, Kit. SCF exists as noncovalently associated dimer at concentrations where it has been possible to study its quaternary structure; it stimulates dimerization and autophosphorylation of Kit at the cell surface. We have used recombinant versions of human SCF and human Kit extracellular domain (sKit) to study SCF-Kit interactions. By size exclusion chromatography, plus various physical chemical methods including light scattering, sedimentation equilibrium, and titration calorimetry, we demonstrate the formation of complexes containing a dimer of SCF (unglycosylated SCF1–165) plus two molecules of sKit. The concentrations of SCF and sKit in these studies were in the range of 0.35–16.2 μM. The data are analyzed and discussed in the context of several possible models for complex formation. In particular, the sedimentation data are not consistent with a model involving cooperative binding. The Kd estimate for SCF-sKit interaction, obtained by sedimentation equilibrium, is about 17 nM at 25 °C. With glycosylated SCF1–165, the Kd is considerably higher.

Stem cell factor (SCF)1 is encoded by the steel (Sl) locus in the mouse (1–3), and is the ligand for the tyrosine kinase type receptor, Kit (1, 3, 4). Kit in turn is encoded by the dominant receptor, Kit (1, 3, 4). Kit in turn is encoded by the dominant

* The abbreviations used are: SCF, stem cell factor; CHO, Chinese hamster ovary; PBS, phosphate-buffered saline; sKit, soluble Kit (extracellular domain).
kinase type receptors appear to be dimeric, it is reasonable to suggest that this property may be important for mediating receptor dimerization within this family. However, this has not been definitively shown. Lev et al. (31) have proposed and discussed a model for SCF-mediated Kit dimerization in which it is not essential that SCF be dimeric when bound to Kit, and the same group has shown that Kit-Kit interactions play a role in Kit dimerization (43).

In the present work, we have used recombinant human soluble SCFs and recombinant human sKit (Kit extracellular domain) to study the stoichiometries and affinities of SCF-Kit interactions by a variety of physical chemical methods. We demonstrate the formation of a complex containing two molecules of SCF monomer plus two molecules of Kit extracellular domain.

**EXPERIMENTAL PROCEDURES**

Materials—E. coli-derived recombinant human SCF1-165 (unglycosylated) and CHO cell-derived recombinant human SCF1-165 (glycosylated) were prepared as described previously (43); their concentrations were determined using ε\textsubscript{280} of 0.536 and 0.540 liters/g cm\textsuperscript{-1}, respectively; these values were calculated from Trp, Tyr, and cystine composition (45). For the CHO cell-derived human SCF1-165, the ε\textsubscript{280} and any mass/volume concentrations given are representative of the polyepitope portion only (i.e. excluding attached carbohydrate). Molecular weight values of 18,656 and 18,525 (for monomers) or 37,313 and 37,051 (for dimers) (formula weights, calculated from amino acid sequence; note that the E. coli-derived SCF has a methionine at position 1 (44)) were used along with mass/volume values to arrive at molar concentrations of E. coli-derived or CHO cell-derived (respectively) SCF. Such concentrations therefore refer to SCF monomer in some cases and to SCF dimer in other cases, as indicated.

\textsuperscript{125}I-labeled E. coli-derived recombinant human SCF of specific radioactivity 61 mCi/mg was prepared by the lactoperoxidase method (46). 12.5 μCi (labeling efficiency 2.3% by weight) of the labeled SCF was mixed with 2.4 mg of unlabeled E. coli-derived human SCF such that the volume of the mixture was 0.4 ml. This solution was applied to a Superose 6 size exclusion column (1 × 29 cm; Pharmacia Biotech Inc.) equilibrated with PBS. Eluting fractions were collected, and those corresponding to the single peak of A\textsubscript{280}-absorbing material, representing SCF, were pooled to give a solution of SCF at 1.71 mg/ml and about 6 million cpm/ml.

CHO cell-derived recombinant human sKit was expressed and purified as described (38). Based on the expressed sequence and the expected processing of the signal sequence, the material represents amino acids 24–520 according to the numbering of Yarden et al. (29), i.e. it corresponds to the entire extracellular domain of Kit. Note that the 4-amino acid insert (residues 510–513) characteristic of the Kit A isoform is present. The material is heavily glycosylated, with mostly N-linked carbohydrate but some O-linked carbohydrate as well (30). Mass/volume concentrations for the sKit were determined using an ε\textsubscript{280} of 1.185 liters/g cm\textsuperscript{-1}, which is a value calculated from Trp, Tyr, and cystine composition (45); thus these concentrations represent the sKit polypeptide only, exclusive of carbohydrate. Molar concentrations of sKit were determined from mass/volume values using a molecular weight value of 55,815 (calculated from amino acid sequence of the polypeptide).

Size Exclusion Chromatography—Size exclusion chromatography of SCFs, sKit, and incubated mixtures of SCFs and sKit was carried out using a Superose 6 column (1 × 29 cm; Pharmacia) equilibrated with PBS at 4 °C at a flow rate of 0.25 ml/min. A\textsubscript{280} of eluting material was monitored with a Pharmacia UV-M monitor.

Laser Light Scattering/Size Exclusion Chromatography—The online light scattering/ chromatography system used three detectors in series: an absorbance monitor at 280 nm (Knauer A293), a laser light scattering detector (Wyatt Mini-Dawn), and a reductive index detector (Polymer Laboratory, PLRI). A Superose 200 column (1 × 30 cm; Pharmacia) equilibrated with PBS was used at room temperature with a flow rate of 0.5 ml/min. Because sKit is a glycoprotein, its refractive increment is unknown, and its total molecular weight cannot be determined from the light scattering data. However, as we have shown previously, polypeptide molecular weights (without the carbohydrate contribution) can be determined by combining the signals from all three detectors and using the extinction coefficient of the polypeptide alone (47, 48). Calibration was accomplished using ribonuclease, ovalbumin, and bovine serum albumin monomer (Sigma) as molecular weight standards.

**RESULTS**

Size Exclusion Chromatography—The stoichiometry of complex formation between SCF and sKit was investigated by mixing SCF and sKit in different molar ratios, incubating, and analyzing complex formation by size exclusion chromatography. The results are given in Fig. 1 for E. coli-derived SCF and sKit. It can be seen that the presence of SCF along with sKit results in the appearance of an early eluting species that must represent the sKit-SCF complex. (SDS-polyacrylamide gel electrophoresis confirmed that both sKit and SCF were part of this species (data not shown).) When sKit and SCF dimer are present at a 4 to 1 molar ratio during the incubation, integration of the chromatogram shows half of the sKit remaining present in free form and the other half as complex (Fig. 1, chromatogram 6); note in chromatogram 6 that the absorbance of the peak representing complex is somewhat greater than that for the free sKit by an amount representing the SCF associated with the sKit. No free SCF is apparent. When sKit and SCF dimer are present at molar ratios of 2 to 1, 1 to 1, and 0.5 to 1, the size exclusion chromatography results show that all sKit is present as complex. Again in each case the absorbance of the peak representing complex is greater than that of the free sKit (Fig. 1, chromatogram 1) by an amount representing the associated SCF. In the case of the sKit to SCF dimer molar ratio of 2 to 1 (Fig. 1, chromatogram 5), no free SCF remains; in the case of the sKit to SCF dimer molar ratio of 1 to 1 (Fig. 1, chromatogram 4), half of the total SCF remains free; and in the case of the sKit to SCF dimer molar ratio of 0.5 to 1 (Fig. 1, chromatogram 3), three-quarters of the total SCF remains free. Taken together, these results strongly indicate that at the concentrations of sKit and SCF used, there is the formation of complex containing equimolar amounts of sKit and SCF monomer.

This equimolar stoichiometry was confirmed with the use of \textsuperscript{125}I-labeled SCF. \textsuperscript{125}I-labeled SCF, prepared as described under “Experimental Procedures,” was mixed with unlabeled SCF (E. coli-derived) to give a preparation having specific radioac-
of sKit and E. coli-derived SCF. sKit at 0.4 µg/ml (0.72 µM) was incubated with levels of SCF ranging from 6.6 to 52.4 µg/ml (0.18–1.40 µM of SCF dimer). These represented molar ratios of sKit to SCF dimer of 4, 2, 1, and 0.5 (chromatograms 6, 5, 4, and 3, respectively). Chromatogram 2 represents SCF alone (in the amount used in chromatogram 3), and chromatogram 1 represents sKit alone. Incubations were carried out at room temperature for 30 min, and aliquots (200 µl) were then subjected to size exclusion chromatography using a Superose 6 column as described under "Experimental Procedures." The amounts of eluting materials were quantitated by integration of areas under peaks (data not shown). The arrows at the top of the figure represent (from left to right) void volume of the column and then the elution positions of bovine thyroglobulin (M, 670,000), bovine γ-globulin (M, 158,000), chicken ovalbumin (M, 44,000), horse myoglobin (M, 17,000), and cyanocobalamin (M, 1350). (Note that, as reported previously (19), the SCF elutes unusually early, probably because of an elongated shape.)

Activity of 163 cpm/µg. A mixture of sKit (at 40 µg/ml) and the SCF preparation having molar ratio of sKit to SCF dimer of 1 was prepared, incubated, and subjected to size exclusion chromatography as in Fig. 1. Fractions containing complex were collected and pooled. Values of 2916 cpm/ml for radioactivity and 0.071 for absorbance at 280 nm were obtained for the pool. From the specific radioactivity of the SCF (163 cpm/µg), it could be calculated that SCF was present in the pool at 17.9 µg/ml or 0.96 µM (for SCF monomer). Because the sKit (M, 55,600 and 38,600, respectively, consist-

Fig. 1. Size exclusion chromatography of incubated mixtures of sKit and E. coli-derived SCF. sKit at 40 µg/ml (0.72 µM) was incubated with levels of SCF ranging from 6.6 to 52.4 µg/ml (0.18–1.40 µM of SCF dimer). These represented molar ratios of sKit to SCF dimer of 4, 2, 1, and 0.5 (chromatograms 6, 5, 4, and 3, respectively). Chromatogram 2 represents SCF alone (in the amount used in chromatogram 3), and chromatogram 1 represents sKit alone. Incubations were carried out at room temperature for 30 min, and aliquots (200 µl) were then subjected to size exclusion chromatography using a Superose 6 column as described under "Experimental Procedures." The amounts of eluting materials were quantitated by integration of areas under peaks (data not shown). The arrows at the top of the figure represent (from left to right) void volume of the column and then the elution positions of bovine thyroglobulin (M, 670,000), bovine γ-globulin (M, 158,000), chicken ovalbumin (M, 44,000), horse myoglobin (M, 17,000), and cyanocobalamin (M, 1350). (Note that, as reported previously (19), the SCF elutes unusually early, probably because of an elongated shape.)

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Size exclusion chromatography experiments were done using a system with refractive index and absorbance detectors in addition to the light scattering detector ("Experimental Procedures"). This allowed us to calculate the molecular weight of the complex (excluding contributions of carbohydrates) with an accuracy of ±5%. Experiments with sKit alone and E. coli-derived SCF alone gave molecular weights of 55,600 and 38,600, respectively, consistent with the formula weights of 55,814 for sKit monomer and 37,313 for SCF dimer. The value for SCF is also consistent with that determined previously (and below) by sedimentation equilibrium (19). We then applied a sample containing 0.60 mg/ml (10.75 µM) sKit plus 0.18 mg/ml SCF (2.2 mol of sKit/mol of SCF dimer), which produced a large peak corresponding to the sKit-SCF complex and a small peak of free sKit.

As we have described previously (49), to calculate the polypeptide molecular weight of the complex we need its extinction coefficient, which depends on the (assumed) stoichiometry. We therefore used an approach in which the calculated molecular weight is deemed valid only if it is consistent with the stoichiometry assumed. If we assume the complex contains one sKit plus two SCF monomers (one SCF dimer), the data imply M, = 163,000, which is inconsistent with the theoretical value of 93,128. With an assumed stoichiometry of two sKit plus four SCF monomers (two SCF dimers) for the complex, the light scattering data give M, = 163,000, again inconsistent with the theoretical value of 186,256. In contrast, when we assume a stoichiometry of two sKit plus two SCF monomers (one SCF dimer), the calculated M, of 149,000 agrees very well with the theoretical value of 148,943. We therefore conclude that the complex indeed contains two sKit and two SCF monomers (one SCF dimer).

Size Exclusion Chromatography of sKit/CHO Cell-derived SCF Complexes—Fig. 2 summarizes size exclusion chromatography experiments identical to those of Fig. 1 except that CHO cell-derived SCF (glycosylated) was used instead of E. coli-derived SCF (nonglycosylated). Note that CHO cell-derived SCF alone elutes earlier than E. coli-derived SCF, but the apparent complex elutes later than that observed with E. coli-derived SCF (Fig. 1) and elutes increasingly later as the initial amount of SCF becomes less. As noted in the Introduction, prior data have indicated that the mitogenic potency and receptor binding affinity of CHO cell-derived SCF are somewhat lower than those of E. coli-derived SCF. The likely explanation for the data of Fig. 2 is that the affinity of CHO cell-derived SCF for sKit is sufficiently low that during the running of the size exclusion columns, dissociated SCF is constantly being separated away from the sKit. In other words, there is a shifting equilibrium during the running of the columns such that the absorption peaks do not represent stable complexes held together with high affinity (as is the case with sKit and E. coli-derived SCF).

Sedimentation Equilibrium of SCF Alone and sKit Alone—Before studying the sedimentation behavior of complexes between sKit and SCF, we characterized each protein by itself. Data (not shown) for E. coli-derived SCF at three loading concentrations (500, 250, 125 µg/ml) and three rotor speeds give an excellent fit to a single species. Using a calculated protein partial specific volume of 0.7433 ml/g (50) and the
measured solvent density of 1.00399 g/ml for PBS, these data imply a molecular weight of 37,360 with a 95% confidence interval from 36,700 to 38,020, which includes the formula weight of 37,313 for an SCF dimer. These data confirm the weight of 37,360 with a 95% confidence uncertainty being due to that in the carbohydrate partial specific volume to derive a carbohydrate weight for CHO cell-derived SCF of 16,900 [14,900–18,100], and a total molecular weight of 53,900 [51,900–55,100], with most of the uncertainty being due to that in the carbohydrate partial specific volume. Again, these molecular weight values are very close to those reported previously for the CHO cell-derived SCF (19).

The sKit behaved as an ideal single monomeric species, with no appreciable self-association at concentrations up to about 1 mg/ml. Using the polypeptide formula weight of 55,815 and partial specific volume of 0.732 ml/g, the data give a carbohydrate weight for CHO cell-derived SCF of 18,900 [15,900–21,200], and a total molecular weight of 74,800 [71,700–77,000]. A second, independent set of data for sKit gave total weight of 75,200 [72,400–77,200], showing excellent reproducibility of the results.

Sedimentation Equilibrium of sKit Plus SCF Mixtures—Sedimentation equilibrium experiments were performed on mixtures of sKit and E. coli-derived SCF over a range of sKit:SCF molar ratios, total concentrations, and rotor speeds in order to verify the binding stoichiometry and to quantitate the strength of the bonding interactions. These studies confirmed the light scattering conclusions that complex containing two receptors plus two SCF monomers (one SCF dimer) can be formed. Fig. 3 shows sedimentation data at 8,000 rpm for a sample with 2 mol of sKit/mol of SCF dimer (Fig. 3, diamonds) at relatively high protein concentrations (30-400 µg/ml). For such a plot (ln[A] versus [radius]²/2), a single species will give a straight line whose slope is proportional to the molecular weight of the species. The solid line shown in Fig. 3 indicates the slope expected for a 2:2 complex, and the dashed line indicates the slope for a 1:2 complex calculated from sedimentation equilibrium data for the separate components. This comparison clearly indicates that this sample contains primarily the 2:2 complex. Also shown in Fig. 3 are data for a sample with the same sKit loading concentration but having 1 mol of sKit/mol of SCF dimer (Fig. 3, squares). The significantly lower slope indicates that excess ligand leads to a rapid fall off in the fraction of 2:2 complexes in favor of 1:2 complexes. Moreover, these data (as well as additional data at lower sKit to SCF ratios) show no evidence of 2:4 complexes (two sKit plus four SCF monomers (two SCF dimers)).

Further quantitative analysis of these sedimentation equilibrium data requires fitting to an appropriate binding model. Our size exclusion chromatography, light scattering, sedimentation equilibrium, and titration calorimetry (next section) studies all indicate that the fundamental interaction stoichiometry is one sKit per SCF monomer and that the largest complex is the 2:2 complex, so we need only consider models that are consistent with this stoichiometry. Fig. 4 schematically illustrates four such binding models. Because at the protein concentrations used in these studies SCF will be essentially completely dimeric, in the first three of these models SCF dimer is treated as a single species with two binding sites for Kit (these are the models used previously to treat the interactions of neurotrophins with the receptors TrkB and TrkC (49)). In Model 1, these two binding sites per SCF dimer have fixed, 

The values in square brackets are 95% confidence intervals.
equivalent affinities. In Model 2, the two binding sites have fixed inequivalent affinities. Model 2 is included for completeness, but because there is no reason to expect that a symmetric SCF dimer will present inequivalent sites, this was not considered an appropriate model for this system. In Model 3, the two binding sites are initially equivalent, but they interact with either positive or negative cooperativity to alter the affinity for binding the second receptor. It is important to note that in this model the actual mechanism of cooperativity is irrelevant; for example, it could arise either through creation of a receptor-receptor interaction site, as has been proposed (31, 43), or through changes in SCF conformation or other mechanisms. Lastly, in Model 4 we allow for a finite dissociation of SCF into monomers, while keeping fixed the intrinsic interaction between Kit and an SCF monomer. That is, there is no cooperativity in this model, and the monomer ↔ dimer equilibrium of SCF is assumed to be independent of whether Kit is bound.

Fig. 5 shows the results of a simultaneous fit of Model 1 to data for sKit plus E. coli-derived SCF mixtures made up at both the two sKit per SCF dimer and one sKit per SCF dimer molar ratios, and at three different rotor speeds. This fit is excellent and returns an intrinsic dissociation constant for each site of 17 [12–21] nM. As implied by Fig. 3, these data fit poorly (with about 10-fold higher variance (not shown)) to a model having only one sKit binding per two SCF monomers (one SCF dimer).

Indeed, the fitted parameters imply that, averaged across the cell, the sample made at a molar ratio of two sKit per SCF dimer shown in Fig. 3 has 83% of its absorbance from the 2:2 complex, 11% from the 1:2 complex, 6% from free sKit, and 0.2% from free SCF, whereas the sample made at a molar ratio of one sKit/SCF dimer has 52% of its absorbance from the 2:2 complex, 43% from the 1:2 complex, 1% from free sKit, and 4% from free SCF. A second, independent set of experiments gave equivalent results, returning an intrinsic dissociation constant of 17 [14–20] nM.

We have also examined fits to the cooperative binding model, Model 3. Because an examination of model data showed that
the distribution of species is more sensitive to cooperativity when there is a larger excess of SCF in the samples, these fits included samples made at molar ratio of 0.5 sKit/SCF dimer. With these data sets, the cooperative model does not improve the fit over a fit to Model 1, and it can be concluded with 95% confidence that the dissociation constant for the second sKit on an SCF dimer is between 0.87 and 1.26 times that of the first. We therefore conclude that there is no binding cooperativity in this system, and we emphasize that this conclusion is based on data at high protein concentration and thus is free of any influence from possible dissociation of SCF dimers.

Model 4, which incorporates the possibility of appreciable dissociation of SCF dimer to monomers, can also be considered. Although our sedimentation data show no evidence for dissociation of SCF dimers, they also cannot rule out a monomer → dimer $K_d$ of the order of a few nanomolar or less. We therefore used model 4 to verify that such a $K_d$ value would not significantly affect our estimates of the $K_d$ for SCF association with sKit.

Lastly, we have also used sedimentation equilibrium to compare the affinities of sKit for glycosylated and nonglycosylated SCFs. Analysis of 12 data sets for sKit plus CHO cell-derived SCF at molar ratios of two sKit per SCF dimer and at one sKit per SCF dimer using Model 1 gave a $K_d$ of 590 [540–630] nM, which implies a 35-fold weaker binding than that for E. coli-derived SCF. The sedimentation data therefore confirm the weaker binding suggested by the size exclusion chromatography studies. Because SCF molecules with different glycosylation have different affinities (14), the 590 nM $K_d$ value determined here represents an average value. However, it is likely that the data analysis does not weight molecules of different affinity equally, so this value probably does not represent a true overall average affinity.

**Titration Calorimetry—**The binding of two sKit molecules per two SCF monomers (one SCF dimer) was also confirmed by titration calorimetry. Fig. 6 (top) shows the results for additions of E. coli-derived SCF to a solution of sKit. The large exothermic response due to binding ends after 7–8 injections (at which point total SCF corresponds to about 0.5 mol of SCF dimer/mol of sKit), followed by a constant small response due to a small mismatch of buffers and/or a small temperature difference between buffers. After subtraction of this constant heat per injection, the heat per injection data could be fitted well to Model 1 (Fig. 6, bottom), returning a stoichiometry of 2.03 [1.94–2.11] sKit binding sites/two SCF monomers (one SCF dimer) with a dissociation constant of 60 nM and Δ$H$ of −13.0 kcal/mol for the binding of each sKit. The estimated dissociation constant, 60 [17–130] nM, has a large uncertainty but is consistent with that found by sedimentation equilibrium.

**DISCUSSION**

By a variety of physical chemical methods, including size exclusion chromatography, light scattering, sedimentation equilibrium, and titration calorimetry, we have clearly shown that SCF and sKit can form a stable 2:2 complex (i.e. two sKit per two SCF monomers or two sKit per SCF dimer). Our studies have utilized recombinant soluble forms of human SCF and human Kit.

The interactions that we describe and the conclusions that we reach provide insight into the possible nature of SCF-Kit interactions in vivo. The 2:2 complex formation clearly provides a mechanism for Kit dimerization, which is known to occur in vivo and to be required for initiation of signal transduction (21, 31, 51). However, because all of our data is obtained in vitro, we cannot definitely state that such a complex would transmit or initiate biological signaling in vivo, and, as always, there are differences between in vitro and in vivo situations. As mentioned in the Introduction, both SCF and Kit are present in membrane-bound as well as soluble (extracellular domain) forms in vivo, and interactions involving the former may not be very well represented by our studies; the kinetics and energetics of interactions at surfaces will differ from those in solution, and our studies obviously would not reflect any interactions that might involve transmembrane or intracellular portions of the receptors. However, the data presented here should be relevant to the interactions that might occur in vivo.

**Fig. 6. Titration calorimetry of SCF binding to sKit.** The upper panel shows the heat evolved (negative signals) as a 500 μM solution of SCF dimer was titrated (20 injections of 3 μl spaced 8 min apart) into the calorimeter cell containing 1.36 ml of a 16.2 μM solution of sKit. The integrated heats from each injection are shown as the data points in the lower panel, after subtraction of the small constant heat per injection apparent in injections 10–20. The solid curve in the lower panel is the best fit to a binding model in which SCF has an unknown number of identical binding sites for sKit. The best fit values imply that there are 2.03 sites/two SCF monomers (one SCF dimer) with a dissociation constant of 60 nM and Δ$H$ of −13.0 kcal/mol.

Nevertheless, our studies suggest that the dimeric nature of SCF is important to the mechanism of Kit dimerization. This would differ from cases of monomeric ligands that can mediate dimerization of their receptors (41, 42). Note that macrophage colony-stimulating factor and platelet-derived growth factor, whose receptors are members of the Type III tyrosine kinase family along with Kit, are covalently held dimers (24–28).

It is important to ask whether our results are in accord with other proposed models for interaction of SCF and Kit and for Kit dimerization. A sequential model for this process was proposed by Lev et al. (31). In this model, the initial binding of Kit to an SCF dimer to form a 1:2 complex is monovalent (i.e. it involves only one of two binding sites/SCF dimer) and has relatively low affinity. This SCF binding triggers a conformational change in Kit that exposes or creates a receptor-receptor...
interaction site (a "receptor dimerization site"). Consequently a second Kit associates, giving rise to an unstable 2:2 complex in which the receptors are held together only by the dimerization site, and only one of the receptors interacts with SCF. Next, the second Kit molecule binds to the second site on the SCF dimer, giving rise to a stable, high affinity 2:2 complex held together via the receptor dimerization site and via the SCF dimer. This model also proposes that when SCF is in great excess over Kit, a single Kit dimer may bind two SCF dimers (each held by only a single interaction with a receptor), forming a 2:4 complex. A key feature of this model, i.e., that receptor dimerization can occur independently of the bivalent nature of the SCF ligand, was invoked primarily to account for the observation that human SCF is able to heterodimerize mouse and human Kit on cells that express both receptors, even though the affinity of human SCF for mouse Kit is very low. This group has proposed that a Kit dimerization site is present in immunoglobulin-like domain 4 of the Kit extracellular domain, because recombinant Kit truncations that lack immunoglobulin-like domain 4 are unable to dimerize (43).

However, this sequential binding model is inconsistent with the behavior we observe for sKit and SCF in significant ways. The model predicts that binding will be highly cooperative, because the addition of a second sKit molecule to a 1:2 complex to form the stable 2:2 complex adds the receptor-receptor binding energy in addition to a second receptor-ligand bond. As discussed above, we find no evidence for cooperativity. In other words, the sequential model predicts that 2:2 complex will persist when ligand is in great excess, whereas we find that even a 2:fold excess of SCF (in terms of monomer) over sKit substantially reduces the proportion of 2:2 complex in favor of 1:2 complex (Fig. 3). Note that reported bell-shaped SCF dose-response curves (43, 51) can be interpreted as being consistent with reported values from different laboratories or techniques, it is important to ask whether the stoichiometry of the interactions and multivalent nature of SCF were explicitly considered, particularly with regard to binding to cell surfaces, where the dimerization of Kit should produce nonlinear Scatchard plots that overestimate the true intrinsic affinity (52).

By size exclusion chromatography and by sedimentation equilibrium, we find that glycosylated SCF has a lower affinity for Kit than does nonglycosylated SCF. This conclusion is consistent with previous data (14) showing lower in vitro biological response potency and weaker binding in a competition type receptor binding assay for glycosylated SCF in comparison with nonglycosylated SCF; the differences were shown to be caused particularly by glycosylation at Asn93 and at Asn65 (14) and presumably represent steric effects of the glycosylation. As mentioned above, we have also shown that soluble SCF and soluble Kit are both present in human serum (mean levels of 177 pm (for SCF monomer) and 5.8 nm, respectively) and, using a Kd value of 1 nm, we had calculated that much (about 85%) of the SCF would be associated with soluble Kit (30). Because native SCF is glycosylated (18), the higher Kd values would lead to a calculation of considerably less SCF association with soluble Kit in the serum. The temperature dependence of Kd noted above would affect such calculations similarly.

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