The Majority of Stem Cell Factor Exists as Monomer under Physiological Conditions

IMPLICATIONS FOR DIMERIZATION MEDIATING BIOLOGICAL ACTIVITY*

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Soluble Escherichia coli-derived recombinant human stem cell factor (rhSCF) forms a non-covalently associated dimer. We have determined a dimer association constant \( K_d \) of \( 2-4 \times 10^7 \) M\(^{-1}\), using sedimentation equilibrium and size exclusion chromatography. SCF has been shown previously to be present at concentrations of approximately 3.3 ng/ml in human serum. Based on the dimerization \( K_d \) greater than 90% of the circulating SCF would be in the monomeric form. When \( ^{125}\)I rhSCF was added to human serum and the serum analyzed by size exclusion chromatography, 72–49% of rhSCF was monomeric when the total SCF concentration was in the range of 10–100 ng/ml, consistent with the \( K_d \) determination. Three SCF variants, SCF(F63C), SCF(V49L,F63L), and SCF(A165C), were recombinantly expressed in Escherichia coli, purified, and characterized. The dimer \( K_d \) values, biophysical properties, and biological activities of these variants were studied. Dimerization-defective variants SCF(F63C)S-CH\(_2\)CONH\(_2\) and SCF(V49L,F63L) showed substantially reduced mitogenic activity, while the activity of the Cys\(_{165}\)-Cys\(_{165}\) disulfide-linked SCF(A165C) dimer was 10-fold higher than that of wild type rhSCF. The results suggest a correlation between dimerization affinity and biological activity, consistent with a model in which SCF dimerization mediates dimerization of its receptor, Kit, and subsequent signal transduction.

Stem cell factor is a cytokine that is active toward early hematopoietic cells and also plays roles in gametogenesis, melanogenesis, and mast cell function. Its biological and other properties have been extensively reviewed (1, 2). It is found in both membrane-bound and soluble forms, with the latter being derived from a membrane-bound form by proteolytic cleavage. The soluble SCF has 165 amino acids.

Both soluble Escherichia coli-derived and CHO\(^1\) cell-derived recombinant human SCF have been reported to be non-covalently associated dimers, as determined by sedimentation equilibrium and size exclusion chromatography at protein concentrations above 0.4 mg/ml (3). In a previous paper (4), we demonstrated that SCF dimer is dissoiable under non-denaturing conditions and the dissociation rate constant \( (k_d) \) of E. coli-derived rhSCF dimer is approximately 1.35 \( \times 10^{-8} \) s\(^{-1}\) at pH 4.8, 25 °C. In the present work, we arrive at a value of 2–4 \( \times 10^{-8} \) M\(^{-1}\) s\(^{-1}\) for the dimer association constant \( (K_d) \) of E. coli-derived rhSCF, based on several approaches including ultracentrifugation and size exclusion chromatography at low SCF concentrations. Since the SCF concentration in human serum has been found previously to be a few nanograms/ml (5), the \( K_d \) value suggests that the majority of SCF in serum may be monomeric. We use \( ^{125}\)I-SCF as a tracer added to serum to show that this does in fact appear to be the case.

The binding of ligands to cell receptors, followed by receptor dimerization, is essential for signal transduction by the family of transmembrane receptor tyrosine kinases (6–8). The receptor for SCF on target cells is Kit (see Refs. 1 and 2 for reviews). Kit is a member of the type III receptor tyrosine kinase subfamily, which also includes the M-CSF receptor (Fms), the PDGF receptor, and Flk2 (6, 7). The ligands for each of these receptors can exist as dimers, non-covalently associated in the cases of SCF (3, 9) and Flk ligand (10), and disulfide-linked in the cases of M-CSF (11, 12) and PDGF (13). It is reasonable to assume that ligand dimerization helps to mediate receptor dimerization for each of these ligand/receptor pairs, but a requirement for ligand dimerization has not been definitively shown in any of these cases. For Kit in particular, dimerization was suggested to be independent of the bivalency of the dimeric SCF ligand (14). However, sedimentation equilibrium and size exclusion chromatography indicated that SCF and Kit form 2:2 complexes in vitro (15). The SCF concentrations used in the latter study were higher than those found in vivo.

As an additional approach to testing whether dimerization of SCF helps to mediate dimerization of Kit, three E. coli-derived SCF variants were designed. Based on the genetic and structural homology between SCF and M-CSF (16), Phe\(^{63}\) of SCF appeared comparable to the corresponding Phe\(^{67}\) of M-CSF, which is located in a loop between the B-helix and C-helix. (M-CSF is a four-helix bundle cytokine (16), and SCF almost certainly is as well (17).) The x-ray crystallographic structure of the M-CSF disulfide-linked dimer (16) has shown that Phe\(^{67}\) resides in the M-CSF dimer interface. Phe\(^{63}\) involvement in the dimer interface of SCF was assumed, and two variants with Phe\(^{63}\) substitutions were therefore prepared. The variant SCF(A165C) was also prepared. Characterization of dimer association constant \( K_d \), biophysical properties, and biological activities of these variants give results consistent with the hypothesis that SCF dimerization is important for Kit dimerization.
Experimental Procedures

Materials—Acetonitrile and HPLC-grade water were purchased from Burdick & Jackson. All protein sequencing reagents were obtained from Applied Biosystems Inc. and Hewlett Packard. Trifluoroacetic acid and other chemicals were purchased from J.T. Baker. Asp-N endoproteinase was purchased from Boehringer Mannheim. 4-Hydroxy-α-cyclo- nonecinamic acid was obtained from Aldrich. Molecular weight standards for size exclusion chromatography were purchased from Bio-Rad and those for SDS-PAGE from Novex. CHO-derived recombinant human soluble Kit was expressed and purified as described (18).

Expression of rhSCF and Its Variants in E. coli—The construction of the expression vector for human SCF, and the fermentation conditions for expression in E. coli were reported previously (19, 20). The expressed gene contains an initiating methionine codon followed by the codons for SCF 1–165 (position 1). The SCF variants were prepared by procedures including site-directed mutagenesis of the parent gene with standard polymerase chain reaction techniques, essentially as described (21).

Purification of E. coli-derived rhSCF, Its Variants, and Derivatives—The purification of rhSCF and its variants was performed essentially as described (20). Briefly, the inclusion bodies were harvested after E. coli cell breakage, solubilized in 8 M urea, and the protein refolded and oxidized by diluting into Tris buffer containing 2.5 M urea and reduced glutathione. Subsequently, purification was conducted incorporating the following steps: S-Sepharose, reversed-phase C4, Q-Sepha- rose, and size exclusion chromatography. SCF(F49L,F63L) was purified by the same procedures as for the wild type rhSCF, except that the reversed-phase C4 step was eliminated. Following the same procedures, partially purified disulfide-linked SCF(F63C) was obtained and subsequently reduced and denatured in 40 mM Tris, pH 8, 8 M urea, 0.5 mM dithiothreitol at 37 °C, for 30 min; the reduced SCF(F63C) was then purified by RP-HPLC. The preparation was dried in a vacuum centrifuge, redissolved in 8 M urea, and refolded by diluting 10-fold using 20 mM Tris-HCl, pH 7.4, 8 M urea, 0.5 mM reduced glutathione and 1 mM oxidized glutathione. Monomeric SCF(F63C) was isolated by a final RP-HPLC step, and was then dried down and reconstituted in PBS. The disulfide-linked SCF(F63C) dimer was formed by diluting the purified SCF(F63C) monomer into 20 mM Tris pH 8.0, 1 mM copper sulfate with stirring for 5 min at room temperature. The preparation was concentra- ted and loaded onto a RP-HPLC column. The disulfide-linked SCF(F63C) dimer was recovered, dried, and redissolved in PBS. SCF(F63C)-CH2CONH2 was made by adding iodoacetamide to SCF(F63C) monomer at a molar ratio of 5 to 1 and was purified by RP-HPLC. The purification of SCF(A165C) was similar to that of SCF(F63C) up to the RP-HPLC step after oxidation in the mixture containing reduced and oxidized glutathione. It was isolated as a dis- sulfide-linked dimer (see "Results").

Size Exclusion Chromatography—Size exclusion chromatography of E. coli-derived wild type rhSCF, SCF variants, and the derivatives was performed using Superdex 75 or Superose 12 columns (Pharmacia Bio- tech Inc.; 10 × 30 mm) equilibrated in 10 mM Tris-HCl, 0.1 mM sodium chloride, pH 7.0, or PBS. The chromatography was carried out using a Hewlett Packard 1050 liquid chromatograph. The detection wavelength was set at 215 nm, and the flow rate was 0.7 ml/min.

Size Exclusion Chromatography of Human Serum Containing 125I-rhSCF—125I-rhSCF was supplied by Amersham International Inc. in sodium phosphate buffer containing 0.5 mM bovine serum albumin. It was prepared by direct iodination with sodium [125I]iodide using the chloramine-T method (22) and desalted/buffer-exchanged by size exclu- sion chromatography. Its specific activity was 56 mCi/mg and the concentra- tion 250 μCi/ml. Fifty μl of the preparation was further purified using a Superdex 75 column. The eluate (12.6–14.2 min) corresponding to rhSCF dimer was collected. The concentration of the radiolabeled rhSCF (0.2 μg/ml) was based upon an assumed recovery of 100%. Three μl of the radiolabeled rhSCF was added to 50 μl of human serum alone or along with 0.6, 1.8, or 5.4 ng of unlabeled rhSCF. These preparations were warmed up to 4 °C and then loaded onto a Superdex 75 column equilibrated in PBS (flow rate 0.7 ml/min). Fractions were collected at 0.4-min intervals and measured for radioactivity using a γ scintillation counter.

RP-HPLC of rhSCF—Ten to 15 μg of E. coli-derived wild type rhSCF, SCF variants, or the derivatives were loaded onto a reversed-phase column (Vydac C4, 4.6 × 250 mm) on a Hewlett Packard 1090 HPLC system as described previously (29).

Results

Size Exclusion Chromatography of E. coli-derived Wild Type rhSCF—When E. coli-derived rhSCF was analyzed by size exclusion chromatography (Superdex 75), a single symmetric peak was seen at higher loading concentrations. As the rhSCF loading concentration was lowered to 0.12 mg/ml, a second peak, eluting after the main peak, began to appear (Fig. 1, A and B). The main peak eluted at 13.8 min, while the second peak eluted at 15.9 min. The molecular weight of the main peak was determined by light scattering to be about 37,600, which corresponds to the molecular weight of the E. coli-derived rhSCF dimer. The second peak, which eluted in the same position as SCF(F63C)-CH2CONH2 monomer (see below in Fig. 5), represented the rhSCF monomer. The rhSCF dimer and mon-omer both elute somewhat earlier than would be expected for globular proteins having these molecular weights (compare.
elution positions of molecular weight standards). As noted previously (3), this result suggests that SCF has an elongated shape.

The calculated percentages of rhSCF monomer in the 0.12, 0.24, and 0.36 mg/ml SCF samples were 2.02 ± 0.20%, 1.45 ± 0.14%, and 1.16 ± 0.11%, respectively. Thus, the percentage of the monomer population increases as the loading concentration of rhSCF decreases. The apparent $K_a ([\text{dimer}] / [\text{monomer}]^2$ was calculated to be $1.85 \times 10^8$ M$^2$.

Equilibrium Ultracentrifugation of E. coli-derived Wild Type rhSCF—The association behavior of rhSCF in solution was studied by sedimentation equilibrium. Because of the low protein concentration needed for detection of the rhSCF monomers, several protein concentrations ranging between 0.02 and 0.5 mg/ml were studied and the absorption scans were taken at 215, 230, and 280 nm. Data sets collected from various loading concentrations and various centrifugation speeds were normalized and fit to different self-association models. For rhSCF solutions with concentration equal to or greater than 0.1 mg/ml, the data fit well to an ideal dimer system. As the protein concentration was decreased, the data fit very well to an ideal (monomer-dimer) system with a dimer association constant ($K_a$) of $2.4 \times 10^8$ M$^{-1}$ (Fig. 2).

Size Exclusion Analysis of Human Serum after Addition of $^{125}$I-rhSCF—Since the concentration of SCF in human serum averages about 3.3 ng/ml (5), it can be calculated from the above estimates of $K_a$ for rhSCF dimerization that a substantial amount of the serum SCF could be monomeric. As another approach to determining whether some SCF at physiologic concentrations might exist as monomers, $^{125}$I-rhSCF was added to human serum and then size exclusion chromatography was performed on a Superdex 75 column. The radiolabeled rhSCF was added to human serum alone, or along with increasing amounts of unlabeled rhSCF. The final rhSCF concentrations were approximately 12, 24, 48, or 120 ng/ml, respectively. A significant amount of SCF monomer was detected in the human serum preparations (Fig. 3). The ratio of the integrated area of monomer peak (peak b) to that of dimer peak (peak a) decreased as the total SCF concentration increased. More than 49% of the SCF was detected as monomer at all the concentrations (Table I). The same results were obtained when the experiment was done with human serum albumin, to which $^{125}$I-rhSCF was added. An additional peak, called peak c, which eluted earlier than the dimer peak, also appeared in the experiment. Since there is a considerable amount of soluble Kit extracellular domain (sKit) in human serum (26), it seemed possible that peak c could represent the SCF-sKit complex. However, complexes of CHO cell-derived human sKit with rhSCF (15) elute earlier than peak c (data not shown). Peak c may result from nonspecific binding of rhSCF to human serum.
components or human serum albumin. These results indicate that the majority of SCF may exist as monomer at the physiological concentration of a few nanograms/ml. The $K_a$ derived from the percentage of monomer obtained at various total rhSCF concentrations is shown in Table I. This $K_a$ agrees with the $K_a$ obtained by sedimentation equilibrium studies, and by the size exclusion chromatographic studies with purified, unlabeled rhSCF described above.

**Characterization of SCF(F63C) and Its Derivatives**—SCF(F63C) and its derivatives (Cys$^{63}$-Cys$^{63}$ disulfide-linked SCF(F63C) dimer and SCF(F63C)-S-CH$_2$CONH$_2$ (carboxyamidomethylation at Cys$^{63}$ position) were obtained as described under “Experimental Procedures.” The preparations were essentially pure and homogeneous, as shown by RP-HPLC and SDS-PAGE (Fig. 4A). Comparing the Asp-N endoproteinase peptide maps of the variant and its derivatives to that of wild type rhSCF (Fig. 4B), the peptides d, e, and f each had the same N-terminal sequence except for position 63. Peptide f had twice the theoretical average mass (Table II), indicating that SCF(F63C) dimer formed an interchain disulfide at position Cys$^{63}$. The mass of peptide d was 57 units higher than the theoretical average mass, indicating that the sulphydryl group of Cys$^{63}$ in SCF(F63C) remained free and the sulphydryl group of Cys$^{63}$ in SCF(F63C)-S-CH$_2$CONH$_2$ had been carboxyamidomethylated.

**Fig. 2.** Sedimentation equilibrium data for *E. coli*-derived wild type rhSCF. The data are for samples run at 26,000, 20,000, and 16,000 rpm. The absorbance at 215 nm is plotted versus $(r^2 - r_o^2)/2$, where $r$ is the radius and $r_o$ is the radius at the reference point of the sample. The upper panel shows the raw data points (symbols) and the fitted curves (solid lines). The model fits an ideal monomer-dimer system. The lower panel represents residuals of the fit. The root-mean-square value is 0.005.

**Fig. 3.** Size exclusion chromatography (Superdex 75) of the human serum samples, to which $^{125}$I-rhSCF was added. The total concentrations of wild type rhSCF are 12 ng/ml (panel A), 24 ng/ml (panel B), 48 ng/ml (panel C), and 120 ng/ml (panel D), respectively, in the human serum samples. Each sample contained 12 ng/ml $^{125}$I-rhSCF. Peak a, which elutes at a retention time of approximately 13.6 min, represents rhSCF dimer; peak b, which elutes at a retention time of approximately 15.8 min, represents rhSCF monomer; and peak c probably represents rhSCF nonspecifically bound to components in human serum. Fractions were collected at 0.4-min intervals and measured for radioactivity.
Upon analysis by size exclusion chromatography (Superose 12), SCF(F63C) and SCF(F63C)-S-CH$_2$CONH$_2$ eluted at 19.4 and 19.5 min (Fig. 5), which corresponds to the position of the wild type rhSCF monomer. The molecular weight of SCF(F63C)-S-CH$_2$CONH$_2$ was determined as 20,900 by light scattering analysis. Sedimentation equilibrium analysis demonstrated that SCF(F63C)-S-CH$_2$CONH$_2$ has a much lower association constant ($K_a$, $1.8 \times 10^6$ M$^{-1}$) than wild type rhSCF (data not shown). These results indicate that SCF(F63C) and SCF(F63C)-S-CH$_2$CONH$_2$ are monomers. SCF(F63C) easily converted to covalent dimer (see below), such that after 24 h in the ultracentrifuge, half of the SCF(F63C) became covalent dimer; thus, the $K_a$ of SCF(F63C) was not determined.

In order to determine if the position 63 variant and its derivatives had substantially altered structure, the solution structures of rhSCF and its variants were compared by CD and fluorescence spectroscopy. The far UV CD signals arise from the asymmetric environments of the aminoterminal residues and thus reflect tertiary structure (28). Using the Greenfield-Fasman equation (27), these spectra suggested more than 40% $\alpha$-helix, as reported previously for wild type rhSCF (3). The near UV CD signals arise from the asymmetric environments of the aromatic residues and thus reflect tertiary structure (28). SCF(F63C) monomer and SCF(F63C)-S-CH$_2$CONH$_2$ had CD spectra identical to that of wild type rhSCF in both the near and far UV regions (Fig. 6B) indicate that wild type rhSCF, SCF(F63C) monomer, and its derivatives contain substantial $\alpha$-helix (27). The Greenfield-Fasman equation (27), these spectra suggested more than 40% $\alpha$-helix, as reported previously for wild type rhSCF (3).

To characterize the folding and association behavior of SCF(F63C) and SCF(F63C)-S-CH$_2$CONH$_2$, we have purified these two disulfide-linked proteins by RP-HPLC (Fig. 8A) and characterized their binding to human serum (5). The mass of the disulfide-linked dimer was significantly decreased, compared to wild type rhSCF (Fig. 6B), indicating 10% less $\alpha$-helix based on calculation by the Greenfield-Fasman equation (28). These results suggest that Phe$^{63}$ resides at the dimer interface, but that the location of the Phe$^{63}$ in the two SCF subunits may not be ideally situated for covalent linkage, such that disulfide bond formation causes structural distortion and the loss of biological activity.

**Characterization of SCF(V49L,F63L) and SCF(A165C)—SCF(V49L,F63L) was purified essentially to homogeneity, as determined by SDS-PAGE. Upon size exclusion chromatographic analysis, the variant eluted in the monomer position at a loading concentration of 0.01 mg/ml (load volume 100 µl), but eluted near dimer position at a loading concentration of 0.3 mg/ml. Equilibrium ultracentrifugation analysis revealed that the association constant ($K_a$) of SCF(V49L,F63L) was $1.47 \times 10^6$ M$^{-1}$. The variant had 30-fold lower mitogenic activity than wild type rhSCF (Fig. 7).

After oxidation and refolding of SCF(A165C), the preparation was further purified by RP-HPLC (Fig. 8A). Peak T42 was monomer and the majority of peak T48.7 was dimer, as determined by non-reducing SDS-PAGE. Comparing the Asp-N endopeptidase peptide maps of these two samples to that of wild type rhSCF (Fig. 8B), peptides g, h, and i each had the same N-terminal sequence except for position 165. Peptide i had twice the theoretical average mass (Table II), indicating that peak T48.7 dimer contained an interchain disulfide at position Cys$^{165}$. The covalent SCF(A165C) dimer had a 10-fold higher biological activity than wild type rhSCF (Fig. 7). The mass of peptide h was 303 units higher than the theoretical average mass, indicating that the T42 species was a glutathione derivative. SCF(A165C)-S-glutathione had biological activity identical to that of E. coli-derived wild type rhSCF (data not shown).

**DISCUSSION**

In this paper, we have demonstrated that the dimer association constant ($K_a$) of E. coli-derived wild type rhSCF is $2-4 \times 10^6$ M$^{-1}$, as determined by equilibrium ultracentrifugation and size exclusion chromatography. Consistent with this $K_a$ value, we have also demonstrated, by using size exclusion chromatography after addition of $^{125}$I-SCF as a tracer, that the SCF monomer is probably the major species in human serum preparations.

Based on Bazan’s prediction of the genetic and structural homology between SCF and M-CSF (17), and the x-ray crystallographic structure of M-CSF (16), the Phe$^{63}$ in SCF probably resides at the dimer interface. Variants with Phe$^{63}$ substituted were therefore studied. Variant SCF(F63C) and its derivative SCF(F63C)-S-CH$_2$CONH$_2$ exist primarily as monomers, as indicated by size exclusion chromatography ($K_a$, $1.8 \times 10^6$ M$^{-1}$ for the derivative). Thus substitution of Phe$^{63}$ with Cys significantly reduces formation of the SCF dimer. Substitution with

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**TABLE I**

Determination of the $K_a$ of wild type rhSCF dimerization from the data in Fig. 3

| SCF concentration added to human serum samples | Corrected SCF concentration (endogenous plus added)$^a$ | SCF concentration after subtraction of non-specific binding to human serum | Percent of monomer$^b$ | $K_a$     |
|-----------------------------------------------|--------------------------------------------------------|---------------------------------------------------------------------|------------------|----------|
| ng/ml                                         | ng/ml                                                  | ng/ml                                                              |                  |          |
| 12                                            | 15                                                     | 10                                                                  | 72               | 5.0 $\times 10^8$ |
| 24                                            | 27                                                     | 18                                                                  | 72               | 2.8 $\times 10^8$ |
| 48                                            | 51                                                     | 34                                                                  | 67               | 2.0 $\times 10^8$ |
| 120                                           | 123                                                    | 89                                                                  | 49               | 2.2 $\times 10^8$ |

$^a$ Human serum contains approximately 3.3 ng/ml SCF (5). Note that the endogenous serum SCF is glycosylated (5). We are assuming that the behavior of $^{125}$I-rhSCF reflects that of total SCF, including the endogenous. Given ability for subunit interchange, this assumption is reasonable.

$^b$ Percent monomer was calculated as the integrated area of peak b (monomer) divided by the sum of the integrated areas of peak b (monomer) and peak a (dimer).
Leu<sup>63</sup> in the variant SCF(V49L,F63L) resulted in a $K_d$ of $1.47 \times 10^5$ M<sup>-1</sup>. Replacing the bulky Phe by Leu could result in unfavorable packing in the hydrophobic dimer interface. We believe that Leu, replacing Val at position 49, would reside in the hydrophobic side of the amphipathic B helix and would not be in the interface of the dimer, again based on the structural analogy to M-CSF. Leu<sup>49</sup> therefore would be less likely to affect SCF dimerization. The conformation of these two variants, determined by circular dichroism and fluorescence, is indistinguishable from that of wild type rhSCF. However, these methods are unlikely to detect small structural alterations.

The disulfide-linked SCF(A165C) dimer would be 100% dimer at all concentrations. Since SCF<sup>1–141</sup> is fully active (29) and is a non-covalently associated dimer as judged by size exclusion chromatography, the Ala<sup>165</sup> almost certainly does not reside in the dimer interface. The C-terminal portion, which would include the disulfide bond Cys<sup>165</sup>-Cys<sup>165</sup> of the SCF(A165C) disulfide-linked dimer, is probably flexible. The phenomenon of dynamic association/dissociation at the dimer interface might still occur in the SCF(A165C) disulfide-linked dimer. However, the association at the interface would be much enhanced because of the interchain disulfide bond (Cys<sup>165</sup>-Cys<sup>165</sup>).

The mitogenic activities of SCF(F63C)S-CH<sub>2</sub>CONH<sub>2</sub> and SCF(V49L,F63L) are 1,300-fold and 30-fold lower, respectively, than that of wild type rhSCF (Fig. 7), while that of SCF(A165C)...

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**TABLE II**

Laser desorption mass spectrometric analysis and sequence analysis of the Asp-N endoproteinase peptides shown in Figs. 4 and 8

| Peptide | Peptide sequence | Observed MH<sup>+</sup> average mass | Theoretical MH<sup>+</sup> average mass | Mass difference between observed and theoretical |
|---------|------------------|--------------------------------------|----------------------------------------|-----------------------------------------------|
| Peptide e | DK<sup>65</sup>SNISEGLSNYSII | 1788.4 | 1787.9 | 0.5 |
| Peptide d | DRC<sup>66</sup>SNISEGLSNYSII | 1801.0 | 1743.9 | 57.1 |
| Peptide f | DRC<sup>66</sup>SNISEGLSNYSII | 3489.5 | 1743.9 | 1745.6 |
| Peptide g | DSRVTSRPFPMLPPVAC<sup>165</sup> | 1816.9 | 1816.2 | 0.7 |
| Peptide h<sup>a</sup> | DSRVSTVFPAMLPPVAC<sup>165</sup> | 2151.2 | 1848.2 | 303.0 |
| Peptide i<sup>b</sup> | DSRVSTVFPAMLPPVAC<sup>165</sup> | 3585.5, 3733.8<sup>b</sup> | 1848.2, 1885.6<sup>b</sup> | 1847.1, 1885.6<sup>b</sup> |

<sup>a</sup> A coeluted peptide is EGLSNYSII<sup>76</sup> (mass data not shown).
<sup>b</sup> Two masses were observed for peptide i. The higher mass represents potassium adduct of the peptide.
is about 10-fold higher. Based on calculation from the \( K_a \) values listed in Table III, the dimer concentration at 50% maximum in the UT-7 mitogenic bioassay is 0.8 ng/ml for the SCF(A165C) disulfide-linked dimer, 0.8 ng/ml for wild type rhSCF, 0.7 ng/ml for SCF(V49L,F63L), and 18 ng/ml for SCF(F63C)S-CH\(_2\)CONH\(_2\). Thus the proliferation bioactivity and \( K_a \) are correlated to some extent, consistent with a hypothesis that dimerization of SCF mediates the mitogenic response. (Models of SCF association with Kit for Kit dimerization and signal transduction are discussed further below.) However, we cannot rule out the possibility that the correlation is coincidental, and that SCF(V49L,F63L) and SCF(F63C)S-CH\(_2\)CONH\(_2\) have lower activity because of other structural alterations that might affect their ability to interact with the receptor.

Since the dissociation rate constant, \( k_d \), for wild type SCF has been determined previously (4), we can estimate the association rate constant \( k_a \) from the \( K_a \) determined in the present work: \( k_a = K_a 	imes k_d = 4 \times 10^4 \) M\(^{-1}\) s\(^{-1}\). Given a soluble SCF concentration of 3.3 ng/ml in human serum (5), the \( K_a \) for SCF dimerization also allows the calculation that about 90% of the

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**Table III**

| | \( K_a \) | \( K_d \) | Total SCF concentration at 50% maximum mitogenic activity | Calculated SCF dimer concentration at 50% of maximum mitogenic activity | Total SCF concentration at 50% inhibition in receptor binding assay |
|---|---|---|---|---|---|
| Wild type SCF | \( 2.7 \times 10^6 \) | \( 3.7 \) nm | 6 ng/ml | 0.8 | 3–7 |
| SCF(V49L,F63L) | \( 1.47 \times 10^5 \) | 6.8 \( \mu \)m | 200 ng/ml | 0.7 | 80 |
| SCF(F63C) | | | >100 \( \mu \)g/ml | 70 |
| SCF(F63C) dimer | | | 10 \( \mu \)g/ml | 18a |
| SCF(F63C)S-CH\(_2\)CONH\(_2\) | \( 1.8 \times 10^3 \) | 0.56 mm | 1800 ng/ml | 900 |

*The rhSCF dimer concentrations were calculated by the equation \( K_a = [\text{dimer}] / [\text{monomer}]^2\). \( X \) is rhSCF monomer concentration (ng/ml) (\( X \) ng/ml \( \times \) (1 g/10\(^6\) ng) \( \times \) (10\(^3\) ml/1 liter) = \( X \times 10^{-6} \) g/liter); \( A \) is total rhSCF concentration (ng/ml) (\( A \) ng/ml = \( A \times 10^{-6} \) g/liter); and \( (A - X) \) is the rhSCF dimer concentration (ng/ml) (\( A - X \) ng/ml = \( (A - X) \times 10^{-6} \) g/liter). The molecular weight of rhSCF monomer is 18,649.\[
\begin{align*}
K_a & = \frac{2 \times 18,649 \text{ g/mol}}{X \times 10^{-6} \text{ g/liter} - (A - X) \times 9.32 \times 10^9 \text{ M}^{-1} \\
& = \frac{(A - X) \times 10^{-6} \text{ g/liter}}{18,649 \text{ g/mol}} \\
K_a X^2 + 9.32 \times 10^9 X - 9.32 \times 10^9 A &= 0 \\
X &= \frac{-9.32 \times 10^9 + \sqrt{(9.32 \times 10^9)^2 + 4 \times K_a \times 9.32 \times 10^9}}{2K_a} \text{ ng/ml}
\end{align*}
\]
SCF would be monomeric, and the size exclusion chromatographic experiments with 125I-SCF added to human serum support this calculation. However, we have shown in vitro that at concentrations where rhSCF is dimeric, a complex is formed with sKit that includes two rhSCF monomers and two sKit molecules (15), and we consider it most likely that SCF dimer mediates Kit dimerization. Models by which SCF could induce receptor dimerization and consequently activate signal transduction are shown in Fig. 9.

In these models, some correlation between $K_a$ for rhSCF dimerization and biological activity, such as that noted above, would be anticipated. The models differ from each other primarily in that each SCF monomer interacts with only one receptor molecule in model I, whereas each SCF monomer interacts with both receptor molecules in model II. In this sense, model II is analogous to the 1:2 growth hormone-growth hormone receptor complex (30), in which a single growth hormone molecule mediates receptor dimerization by interacting with both receptor molecules. It is important to note (e.g. with regard to monomeric SCF which may be present in human serum) that each model allows that an SCF molecule be initially monomeric and still wind up as part of a 2:2 SCF-sKit complex.

It is also noteworthy that reported $K_a$ values for SCF binding to Kit (ranging from $0.6 \times 10^8$ to $2 \times 10^9$ M$^{-1}$, depending on the system; see Refs. 14, 15, 18, 31, and 32) are close to the $K_a$ reported here ($2-4 \times 10^8$ M$^{-1}$) for rhSCF dimerization. This could be considered consistent with a model in which SCF-sKit interactions were relatively strong and sKit-sKit interactions relatively weak or non-existent, such that SCF-SCF interactions determine the apparent $K_a$ for formation of the 2:2 SCF-Kit complex (15). We do not attempt to address the issues...
of conformational changes and cooperative interactions in SCF-Kit complex formation (14, 15, 33).

In summary, the details of SCF-Kit interactions will require further elucidation, but it is apparent that the association and dissociation among SCF monomer, SCF dimer, and Kit are dynamic, and play an important role in the regulation of the development and function of hematopoietic cell lineages and other cells such as mast cells, germ cells, and melanocytes.

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FIG. 9. Models of 2:2 SCF-Kit complex formation mediated by SCF dimerization. In model I, SCF contains an equivalent receptor binding site on each monomer. In model II, each SCF monomer contains two non-equivalent receptor binding sites. The shapes of Kit (R) and SCF monomer (L) are schematic and not intended to imply any specific structural features.
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