Interaction of Recombinant Granulocyte Colony Stimulating Factor with Lipid Membranes: Enhanced Stability of a Water-Soluble Protein after Membrane Insertion

David Collins* and Younsik Cha

Department of Pharmaceutics and Drug Delivery, Amgen, Inc., Mailstop 8-1-A-215, Amgen Center, Thousand Oaks, California 91320

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ABSTRACT: The interaction of recombinant granulocyte colony stimulating factor (rhG-CSF) with lipid vesicles was studied. In the presence of dioleoylphosphatidylglycerol (DOPG) vesicles, the intrinsic fluorescence of rhG-CSF exhibits dramatic changes. In particular, tryptophan fluorescence is greatly enhanced and the emission maximum shifted to lower wavelengths. The presence of DOPG vesicles causes the protein tryptophans to become inaccessible to iodide, a water-soluble quencher of tryptophan fluorescence, yet accessible to quenching via energy transfer to pyrenyl decanoic acid, a lipid-soluble fluorescent probe. The data suggest that rhG-CSF inserts into lipid vesicles composed of DOPG. The driving force for the insertion may be a conformational change induced by the low pH at the lipid–water interface of DOPG vesicles. The DOPG-inserted form of rhG-CSF retains biological activity and shows remarkable stability, even under high-temperature conditions which lead to denaturation of rhG-CSF alone. Membrane insertion of G-CSF may be involved in the in vivo activity of this important cytokine.

Granulocyte colony stimulating factor (G-CSF) is a hematopoietic growth factor which stimulates neutrophil proliferation and activity (Metcalf, 1984; Cohen et al., 1987; Zsebo et al., 1986). A recombinant form of the protein (rhG-CSF) has recently become an important therapeutic agent for the treatment of chemotherapy-induced neutropenia (Souza et al., 1986; Morstyn et al., 1988). rhG-CSF is a nonglycosylated single-chain polypeptide with a molecular mass of 18.5 kDa (Souza et al., 1986).

The structure of rhG-CSF under various conditions has been extensively studied (Nahri et al., 1991; Lu et al., 1992). rhG-CSF is most stable under acidic conditions (Nahri et al., 1991), despite the fact that in the pH range of 2.5–5.0 a conformational change occurs which involves a loosening of the tertiary structure and an increase in α-helical content (Nahri et al., 1991). Such a conformational change is characteristic of the so-called molten globular state (Dolgikh et al., 1981; Ohgushi & Wada, 1983; Bychkova et al., 1988), a condition implicated in the membrane insertion of several proteins (Bychkova et al., 1988; van der Goot et al., 1991). While rhG-CSF is a water-soluble protein, it has been shown to exhibit hydrophobic behavior under certain conditions (Nicola et al., 1983; Lu et al., 1992). In particular, G-CSF binds to phenyl-Sepharose (Nicola et al., 1983), and some folding intermediates are more hydrophobic than the native protein (Lu et al., 1992).

Cytokines such as G-CSF must interact with membrane receptors on their target cells in order to be effective. While much is known about the activity of G-CSF, the exact portion of the molecule involved in receptor binding is unknown (Hill et al., 1993). Recently, it has been shown that some cytokines and growth factors exist in both membrane-bound and soluble forms (Uemura, 1993; Tanaka, 1993; Gray et al., 1983; Lee et al., 1985; Kriegler et al., 1988; Rettenmier et al., 1987; Anderson et al., 1990). In light of these studies and the fact that G-CSF has been shown to exhibit hydrophobic behavior under certain conditions (Nicola et al., 1983; Lu et al., 1992), it was of interest to know if G-CSF could exist in a membrane-bound form.

In the present study, we describe the interaction of rhG-CSF with phospholipid vesicles. rhG-CSF inserts into DOPG, but not DOPC, vesicles. The DOPG-inserted form of the protein shows remarkable stability under conditions which lead to denaturation of rhG-CSF in the absence of DOPG.

EXPERIMENTAL PROCEDURES

Materials. Recombinant human G-CSF (rhG-CSF) was prepared as described (Souza et al., 1986). All phospholipids were obtained from Avanti Polar Lipids (Alabaster, AL). Lipid purity was evaluated using thin-layer chromatography. Pyrenyl decanoic acid was purchased from Molecular Probes (Eugene, OR).

Liposome Preparation. Sonicated unilamellar vesicles (SUV) were prepared by hydrating dry lipid films in water and then sonicating the suspensions to optical clarity using a bath-type sonicator (Laboratory Supplies, Hicksville, NY). The final lipid concentration was 30 mM.

Fluorescence Measurements. rhG-CSF fluorescence was measured using a PTI Alphascan fluorometer. Emission spectra were obtained by exciting rhG-CSF samples at 280 nm (5-nm slit width) and scanning emission from 285 to 420 nm (3-nm slit width). Various lipid:rhG-CSF ratios were achieved by direct addition of lipid vesicles to rhG-CSF. The final rhG-CSF concentration was 0.2 mg/mL. The final pH was between 4.5 and 4.75 in all samples. Samples at other pH values were also prepared by adding small aliquots of 50 mM sodium acetate (pH 3) or 50 mM Mes (pH 7.0).

Iodide quenching experiments were performed by adding aliquots of 4 M KI to rhG-CSF (0.2 mg/mL) or DOPG/rhG-CSF (100:1 mol/mol). The samples contained 1 mM Na_2SO_3 to keep the iodide reduced and prevent formation of

* To whom correspondence should be addressed. Phone: (805)-447-2074. Fax: (805)-498-8674.

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I_2, as described (Lee et al., 1989; Le Doan et al., 1986). The data were analyzed by the Stern–Volmer equation: $F_0/F = 1 + K_Q[Q]$, where F_0 and F are the fluorescence intensities in the absence and presence, respectively, of the quencher (iodide) at concentration [Q]. K_Q is the Stern–Volmer quenching constant. The iodide quenching data were also analyzed using a modification of the Stern–Volmer equation as described (Lehrer, 1971): $F_0/\Delta F = (f_a K_Q[Q])^{-1} + f_a^{-1}$, where F_0 is the fluorescence intensity in the absence of quencher, ΔF is the difference in fluorescence intensity in the absence and presence of quencher, K_Q is the Stern–Volmer quenching constant, and f_a is the fraction of tryptophan fluorescence accessible to the quencher.

Energy transfer from tryptophan to pyrenyl decanoic acid was performed as described (Friere et al., 1983). Small aliquots of pyrenyl decanoic acid in tetrahydrofuran were added to DOPG/rhG-CSF (100:1 mol/mol) or DOPC/rhG-CSF (100:1) suspensions while stirring the samples to promote contact between the fluorescent fatty acid and the lipid vesicles. The final tetrahydrofuran concentration was always below 0.4% (v/v), and did not affect the fluorescence of the samples (data not shown). F and F_0 refer to the fluorescence of the samples in the presence and absence, respectively, of pyrenyldecanoic acid.

Circular Dichroism Measurements. Circular dichroism measurements were made on a Jasco J-720 instrument equipped with a Peltier-type thermostated cell holder and a magnetic stirrer. Circular dichroism at 222 nm was measured using a final rhG-CSF concentration of 80 μg/mL, pH 6.0.

Differential Scanning Calorimetry. Differential scanning calorimetry measurements were made using a Microcal MC-2 calorimeter. Samples of rhG-CSF (1 mg/mL, in water, pH 7.0) or DOPG/rhG-CSF (45:1 mol/mol, in water, pH 7.0) were scanned at a rate of 90 °C/h. Data were stored and analyzed using the Microcal software.

G-CSF Biological Activity Assay. The activity of rhG-CSF was assayed as described (Zsebo et al., 1986), utilizing the G-CSF-dependent uptake of [^{3}H]thymidine by murine bone marrow cells. All activity assays were performed in triplicate.

RESULTS

There are two tryptophan residues in rhG-CSF that are quite sensitive to local environmental conditions (Nahri et al., 1991). Tryptophan fluorescence was measured by exciting samples of rhG-CSF at 280 nm and scanning emission from 285 to 420 nm. The fluorescence spectra of rhG-CSF in the presence and absence of small unilamellar vesicles composed of DOPG are shown in Figure 1. rhG-CSF has an emission maximum at 334 nm in the absence of DOPG vesicles. In the presence of DOPG at a 100:1 lipid:protein (mol/mol) ratio, rhG-CSF tryptophan fluorescence exhibits a blue shift in the fluorescence emission maximum to 327 nm and a dramatic increase in fluorescence intensity. The low wavelength of the fluorescence emission in the presence of DOPG suggests that the tryptophans are in an environment more hydrophobic than in the native protein (Strittmatter & Daily, 1982). The fluorescence shifts depend on the DOPG:rhG-CSF mole ratio (Figure 2A), and the effect seems to require at least 10 DOPG molecules per molecule of rhG-CSF. DOPC vesicles had no effect on either the emission maximum or the fluorescence intensity of rhG-CSF, indicating that no interaction took place with this phospholipid (Figure 2B).

As previously demonstrated (Nahri et al., 1991), rhG-CSF undergoes a conformational change in response to pH which

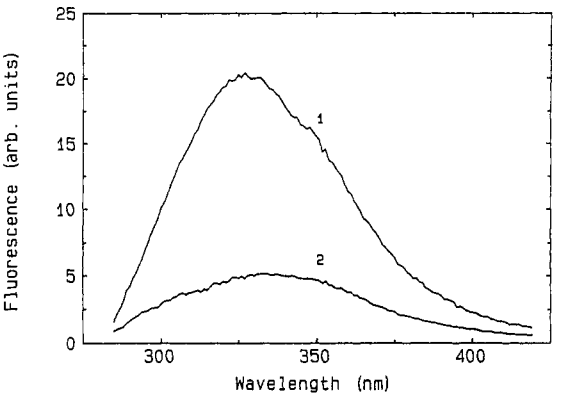

FIGURE 1: Fluorescence emission spectrum of rhG-CSF in the presence (curve 1) and absence (curve 2) of DOPG vesicles. The concentration of rhG-CSF was 0.2 mg/mL. The DOPG:rhG-CSF ratio (curve 1) was 100:1 (mol/mol).

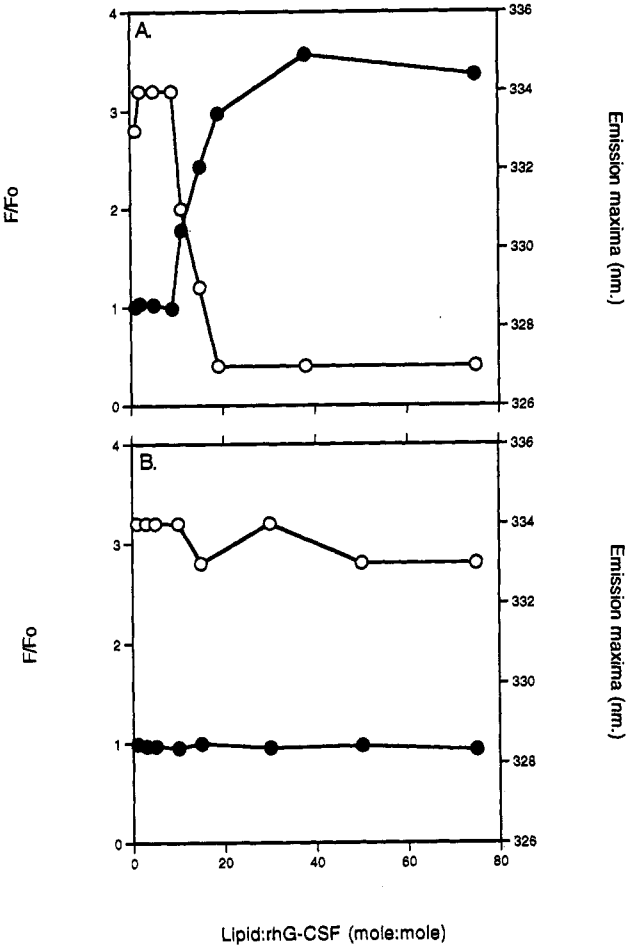

FIGURE 2: Effect of increasing lipid:protein ratio on rhG-CSF fluorescence. F_0 is the initial fluorescence (no lipid), and F refers to the fluorescence after addition of lipid to achieve the indicated molar ratio of lipid to rhG-CSF. F/F_0 (●) and wavelength of maximum emission (○) for mixtures of DOPG/rhG-CSF (A) and DOPC/rhG-CSF (B).

involves a loosening of the protein tertiary structure. This conformational change is easily demonstrated by monitoring the ratio of tyrosine fluorescence [F(Y); emission = 308 nm] to tryptophan fluorescence [F(W); emission = 334 or 327 nm] in response to pH (Nahri et al., 1991). The ratio of F(Y)/F(W) reflects the tyrosine to tryptophan energy transfer and is a sensitive measure of the tertiary structure of rhG-CSF (Nahri et al., 1991). As shown in Figure 3A, rhG-CSF exhibits an increase in the F(Y)/F(W) ratio as the pH is

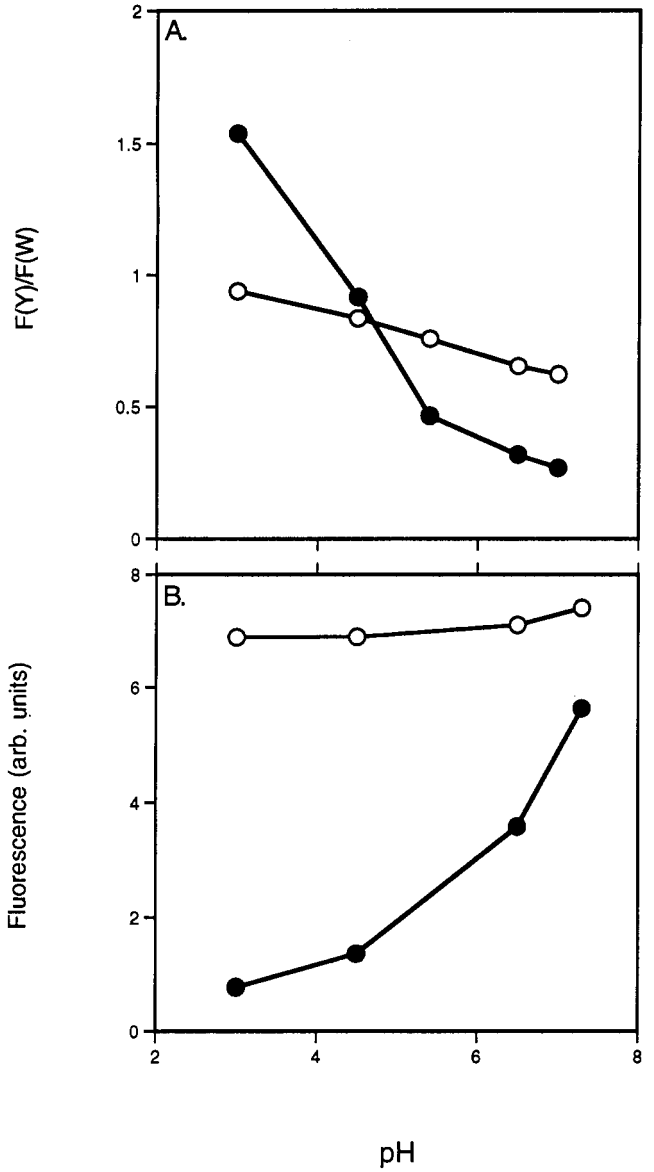

FIGURE 3: Effect of pH on the fluorescence of rhG-CSF (●) and DOPG/rhG-CSF (100:1 mol/mol) (○). (A) Effect of pH on the ratio of tyrosine to tryptophan fluorescence [F(Y)/F(W)]. (B) Effect of pH on tryptophan fluorescence (excitation = 298 nm).

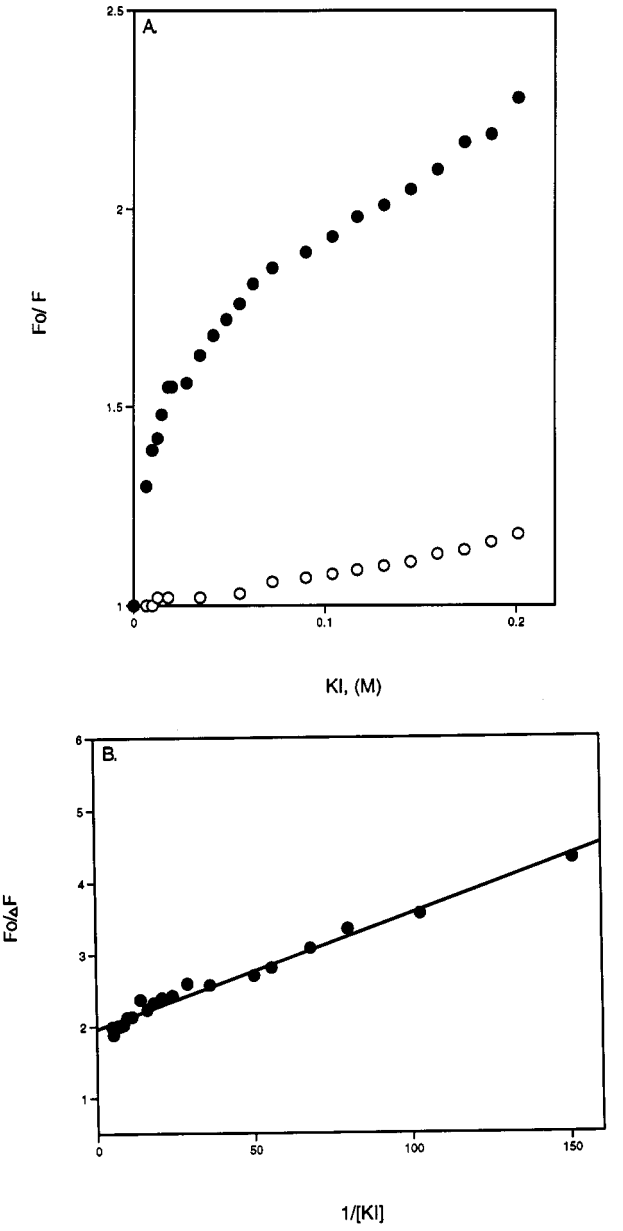

FIGURE 4: (A) Stern–Volmer plots of the quenching of rhG-CSF fluorescence by KI in the absence (●) and presence (○) of DOPG vesicles. Quenching experiments were performed by adding aliquots of KI to rhG-CSF (0.2 mg/mL) and DOPG/rhG-CSF (100:1 mol/mol). (B) Modified Stern–Volmer plot of the quenching of rhG-CSF fluorescence by KI in the absence of DOPG.

decreased from 7.0 to 3.0. This increase is due to dequenching of tyrosine fluorescence as the energy transfer from tyrosine to tryptophan decreases and reflects a loosening of the rhG-CSF tertiary structure. By contrast, DOPG/rhG-CSF samples show a relatively minor increase in the F(Y)/F(W) ratio (Figure 3A). This could indicate that energy transfer and tertiary structure are maintained in the membrane-bound state. Alternatively, these data could result from an absolute increase in tryptophan fluorescence. In order to examine these possibilities, the experiments were repeated using an excitation wavelength of 298 nm to preferentially excite rhG-CSF tryptophan residues (Nahri et al., 1991). As the pH decreases, rhG-CSF exhibits a dramatic decrease in tryptophan fluorescence (Figure 3B). By contrast, the tryptophan fluorescence from DOPG/rhG-CSF samples is insensitive to pH (Figure 3B). The data indicate that the rhG-CSF fluorescence changes seen in the presence of DOPG are due to an increase in tryptophan fluorescence.

To further examine the possibility of rhG-CSF insertion into DOPG membranes, iodide quenching experiments using KI were performed. Iodide is an efficient collisional quencher of tryptophan fluorescence (Lee et al., 1989; Le Doan et al., 1986), but cannot penetrate lipid membranes. Therefore, efficient quenching of tryptophan fluorescence by iodide indicates exposure of the residues to the bulk aqueous solvent while protection from iodide quenching occurs when protein tryptophans are sequestered away from the aqueous solvent. In these experiments, a high DOPG:rhG-CSF ratio was used to ensure interaction (Figure 2A). The fluorescence (excitation = 280 nm; emission = 327 or 334 nm) of rhG-CSF in the absence or presence of DOPG vesicles was recorded for increasing amounts of KI. The Stern–Volmer plots of the data are shown in Figure 4. In the absence of DOPG vesicles, rhG-CSF fluorescence is efficiently quenched by KI. The two tryptophans show different accessibility to iodine as evidenced by the two components in the Stern–Volmer plot of the data (Figure 4A). Analysis of the data using the modified Stern–Volmer equation (Lehrer, 1971) indicates that

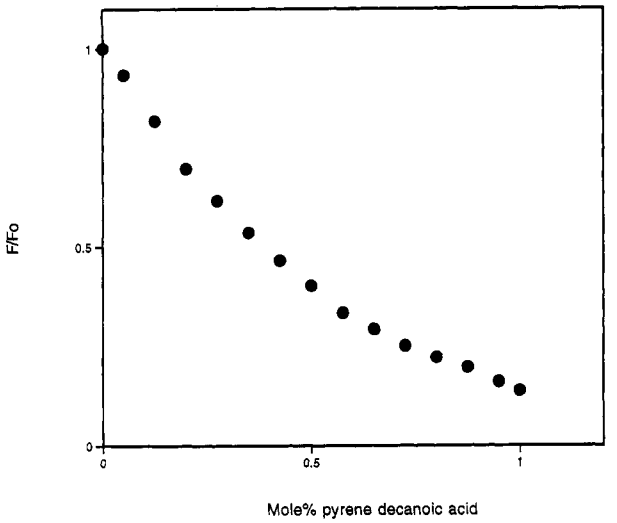

FIGURE 5: Quenching of rhG-CSF tryptophan fluorescence upon addition of pyrenyldecanoic acid. The excitation wavelength was 280 nm, and the emission wavelength was 327 nm. The DOPG:rhG-CSF ratio was 100:1 (mol/mol).

only one tryptophan residue (f_a = 0.5) in rhG-CSF is fully accessible to iodide under these conditions (Figure 4B). In the absence of DOPG, the Stern–Volmer quenching constant (K_Q) for the "iodide–accessible" tryptophan is 125 M^{-1}, as derived from the modified Stern–Volmer plot (Lehrer, 1971) (Figure 4B). In the presence of DOPG, the overall K_Q is decreased to 0.77 M^{-1} (Figure 4A), and the Stern–Volmer plot of the data is linear, indicating that iodide has poor access to both tryptophans. The data show that a tryptophan residue which is iodide-accessible in the absence of DOPG becomes iodide-inaccessible in the presence of DOPG. Therefore, the portion of rhG-CSF containing this tryptophan must be embedded in the DOPG bilayer.

An alternate way to examine the insertion process is to use resonance energy transfer (Stryer, 1978). As previously shown, energy transfer can occur between tryptophan donors and lipid-soluble fluorescent acceptors such as pyrenyl decanoic acid (Friere et al., 1983) or diphenylhexatriene (Le Doan et al., 1983), since the excitation spectrum of these probes significantly overlaps the emission spectra of tryptophan. If the protein inserts into lipid membranes, energy transfer from tryptophan to pyrene will lead to quenching of the tryptophan fluorescence. Figure 5 shows the quenching profile for rhG-CSF in the presence of DOPG (100:1 lipid:protein mol/mol ratio) as a function of added pyrenyl decanoic acid. The quenching occurs at very low pyrenyl decanoic acid concentrations (<1 mol %), so the effect of the fluorescent probe on membrane structure and behavior is minimal. Since pyrenyl decanoic acid can be expected to rapidly partition into lipid bilayers, the present data indicate that rhG-CSF is embedded in DOPG membranes deep enough to allow efficient energy transfer from tryptophan to the pyrene acceptor. Energy transfer was confirmed by examining the excitation spectra of pyrenyl decanoic acid-labeled DOPG vesicles (emission = 380 nm) in the presence and absence of rhG-CSF (data not shown). In the presence of rhG-CSF, an excitation peak is detected at 292 nm (data not shown); this is close to the absorption maximum of tryptophan. In the absence of rhG-CSF, no peak at 292 nm is seen. No excited dimer (excimer) formation was noted, indicating that both the fluorescent acceptor and the inserted rhG-CSF are randomly distributed in the DOPG bilayers (data not shown). No quenching of rhG-CSF fluorescence was observed in the same concentration

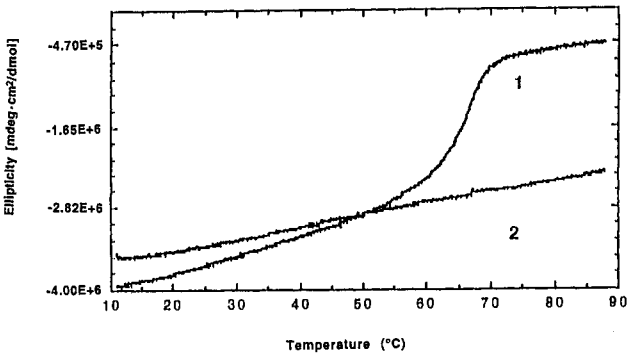

FIGURE 6: Effect of increasing temperature on the CD of rhG-CSF (curve 1) or DOPG/rhG-CSF (140:1 mol/mol) (curve 2). The rhG-CSF concentration was 80 μg/mL in water, pH 6.0. The temperature was scanned from 10 to 90 °C at a rate of 100 °C/h.

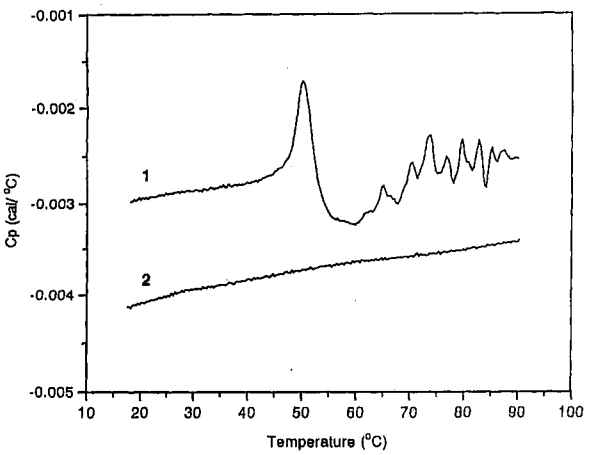

FIGURE 7: Differential scanning calorimetry thermograms for rhG-CSF (curve 1) and DOPG/rhG-CSF (45:1 mol/mol) (curve 2). The concentration of rhG-CSF in the samples was 1 mg/mL (pH 7.0 in water). The scan rate was 90 °C/h.

range of pyrenyldecanoic acid when DOPC vesicles were used instead of DOPG vesicles (data not shown).

The interaction of rhG-CSF and DOPG vesicles exerts a stabilizing effect on the protein, even under conditions (pH >5.0) where rhG-CSF alone is relatively unstable (Nahri et al., 1991; Lu et al., 1992). Temperature-induced changes in α helicity can be followed by measuring circular dichroism (222 nm) as a function of increasing temperatures. The thermally-induced unfolding of rhG-CSF at pH 6.0 is shown in Figure 6. The curve indicates that a fairly sharp transition occurs at ~60–70 °C which leads to a loss of α helicity. After this transition, the rhG-CSF irreversibly precipitates from solution (data not shown). The temperature range of the unfolding is similar to the melting temperature of rhG-CSF at pH 7.0 as determined by differential scanning calorimetry (Figure 7). By contrast, DOPG/rhG-CSF samples show a gradual loss of α helicity with increasing temperature (Figure 6). Unlike rhG-CSF alone, the temperature-induced unfolding of DOPG/rhG-CSF does not appear to be cooperative (Figure 6). This conclusion is supported by the lack of a melting transition by differential scanning calorimetry (Figure 7). Remarkably, DOPG/rhG-CSF samples can recover α helicity after heating to 95 °C and can be repeatedly cycled between 95 and 10 °C with full recovery of helicity upon cooling (Figure 8). rhG-CSF alone under these conditions is irreversibly unfolded and precipitates from solution (Figure 8 and data not shown).

Insertion into DOPG bilayers does not adversely affect the biological activity of rhG-CSF (Table 1). After being heated

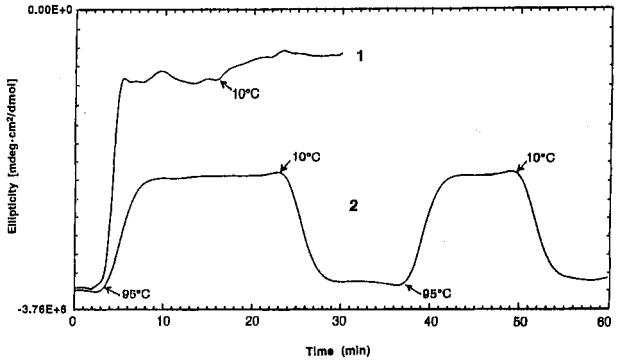

FIGURE 8: Effect of temperature cycling on the CD of rhG-CSF (curve 1) and DOPG/rhG-CSF (140:1) (curve 2). The samples were rapidly heated to 95 °C and cooled to 10 °C as indicated by the arrows. The rhG-CSF concentration in the samples was 80 μg/mL, pH 6.0.

Table 1: Membrane-Inserted rhG-CSF Retains in Vitro Activity

	sp act. (units/mg of protein)
G-CSF	0.66 ± 0.09
G-CSF (heated)[a]	ND[b]
DOPG/G-CSF[c]	0.61 ± 0.11
DOPG/G-CSF[c] (heated)[a]	0.52 ± 0.08

[a] Sample was incubated for 10 min at 85 °C in a water bath prior to performing the in vitro assay (Zsebo et al., 1986). [b] Not detectable. [c] DOPG:G-CSF ratio 50:1 (mol/mol).

to 85 °C for 10 min, rhG-CSF has undetectable activity, and the protein precipitates (Figure 6 and Table 1). After similar treatment, DOPG/rhG-CSF (50:1 mol/mol) retains ~85% of the activity of unheated rhG-CSF and fully recovers secondary structure upon cooling (Figures 6 and 8 and Table 1).

DISCUSSION

DOPG, but not DOPC, enhances and causes a blue shift in rhG-CSF fluorescence. In the presence of DOPG vesicles, a rhG-CSF tryptophan is protected from a water-soluble fluorescence quencher but susceptible to quenching via energy transfer to a hydrophobic fluorescent probe. Taken together, the data show that rhG-CSF can insert into membranes composed of DOPG. The insertion process requires at least 10 DOPG molecules per rhG-CSF (Figure 2A), and this may represent the number of lipids which surround the inserted portion of the protein. By comparison, 14 lipid molecules have been shown to be required to surround the hydrophobic tail of cytochrome b_5 (Friere et al., 1983). To our knowledge, membrane insertion of G-CSF has not been previously demonstrated.

G-CSF appears to belong to a class of proteins which can exist in either a water-soluble or a membrane-bound form. These proteins share the ability to transition into a "molten globular" state which is an unfolded yet compact conformation. Proteins in the molten globular state exhibit secondary structure which is comparable to the native protein, yet they lack rigid tertiary structure (Dolgikh et al., 1981; Ohgushi et al., 1983; Bychkova et al., 1988; van der Goot et al., 1991). This conformation can be detected by comparing the circular dichroism in the far-UV region with the spectra of aromatic side chains (near-UV circular dichroism and fluorescence). The molten globular state exhibits aromatic group spectral changes in the absence of far-UV circular dichroism changes and may be involved in membrane penetration by some proteins (Bychkova et al., 1988; van der Goot et al., 1991). rhG-CSF has been shown to undergo conformational changes in response to decreasing pH which are characteristic of the molten globular state (Nahri et al., 1991). In particular, as the pH is decreased, rhG-CSF exhibits an increase in α helicity while the tertiary structure becomes more flexible. This effect is seen in the range of pH 2.5–5.0 (Nahri et al., 1991).

Negatively charged phospholipids are known to promote a lower surface pH relative to the bulk solvent pH (van der Goot et al., 1991; Wininski, et al., 1986). For example, DOPG vesicles have been shown to exhibit an interfacial pH 1.6–2.0 pH units below the bulk pH (van der Goot et al., 1991). This effect is due to an electrostatic potential which is formed at the surface of the vesicle. Neutral lipids, such as phosphatidylcholine (PC), do not show this effect. Most of the experiments described in this study were performed at acidic pH where rhG-CSF is most stable (Nahri et al., 1991). If the interfacial pH of DOPG vesicles is taken into consideration, the pH experienced by rhG-CSF at the vesicle surface can be expected to be close to 2.5 when the bulk pH is 4.5 and around 5.0 when a bulk pH of 7.0 is used. These values lie within the range of the acid-induced conformational changes described for rhG-CSF [Figure 3 and Nahri et al. (1991)].

The data suggest the following model for the rhG-CSF–DOPG interaction. rhG-CSF binds to DOPG vesicles due to their negative charge and the fact that rhG-CSF is below its p*I* and is positively charged (Nicola et al., 1983; Souza et al., 1986; Nahri et al., 1991). The local pH at the DOPG–water interface induces a conformational change in rhG-CSF, making the protein more flexible and lowering the energy required to fully expose the hydrophobic domain responsible for the insertion.

At the present time, the exact region of rhG-CSF involved in the membrane insertion process is unknown; however, our data suggest that interaction may involve a portion of the AB loop (Hill et al., 1993), a region which consists of amino acid residues 39–72 and includes a tryptophan residue at position 58. We make this hypothesis on the basis of the crystal structure of rhG-CSF (Hill et al., 1993), the iodide quenching data, and the emission spectral changes brought about by the addition of DOPG. In the absence of DOPG, only one rhG-CSF tryptophan is accessible to iodide (Figure 4B). On the basis of the recently derived crystal structure of rhG-CSF, the tryptophan at position 118 is most likely sequestered within the four-helix bundle (Hill et al., 1993) and thus shielded from solvent and external quenchers, such as iodide. Additionally, the fluorescence emission spectra of tryptophan-118 should be blue-shifted relative to those of tryptophan-58. By contrast, tryptophan-58 is located in the AB loop, a relatively exposed region of the protein (Hill et al., 1993). The fluorescence emission from this residue is expected to be red-shifted and less intense relative to that of tryptophan-118. Tryptophan-58 would also seem to be more accessible to iodide quenching than tryptophan-118. Therefore, the blue shift in emission wavelength and the increased fluorescence intensity we observed in the presence of DOPG are most likely due to changes in the environment of tryptophan-58. The decreased iodide accessibility in the presence of DOPG is also likely to be due to membrane insertion of tryptophan-58, since tryptophan-118 is iodide-inaccessible, even in the absence of DOPG. We have also found that both granulocyte macrophage colony stimulating factor (GM-CSF) and porcine somatotropin (PST) are stabilized by insertion into DOPG membranes (unpublished results). Both of these proteins share

structural similarities to G-CSF (Hill et al., 1993), suggesting that membrane insertion may be a common property of this class of cytokines.

Regardless of the exact mechanism of membrane insertion, the interaction of rhG-CSF with DOPG enhances the stability of the protein under conditions where rhG-CSF alone is unstable (Nahri et al., 1991). The basis of this stabilizing effect is unclear at the present time. Insertion of rhG-CSF into DOPG membranes may bury a hydrophobic region of the protein and therefore inhibit protein–protein contacts and rhG-CSF aggregation. The inserted form of the protein may also be held by the membrane in such a way as to allow refolding of the partially unfolded rhG-CSF which is produced upon heating. We are currently exploring these possibilities.

The insertion of rhG-CSF into lipid membranes may have physiological relevance. Several cytokines have been shown to exist in soluble and membrane-bound forms (Kriegler et al., 1988; Anderson et al., 1990; Uemura et al., 1993). Membrane-bound cytokines not only are involved in transmitting signals to their target cells but also may be involved in cell–cell adhesion in inflammation (Uemura et al., 1993). Anchoring inflammatory cytokines, such as G-CSF, to membranes, either by membrane insertion or by binding to proteoglycans on endothelial cells, may also serve to localize the cytokine and the inflammatory response to a specific site in vivo (Uemura et al., 1993; Tanaka et al., 1993). In most cases, membrane-bound cytokines and growth factors contain transmembrane sequences (Uemura et al., 1993; Gray et al., 1983; Kriegler et al., 1988; Rettenmier et al., 1987; Anderson et al., 1990); however, the possibility of membrane-anchoring of soluble cytokines has not been explored. G-CSF represents the first example of membrane insertion by a soluble cytokine which lacks a membrane-spanning region.

The present findings also have definite practical significance. Protein pharmaceuticals are often beset with the problem of poor stability. If the protein in question has the ability to enter an intermediate conformation such as the molten globular state, combining the protein with lipid vesicles or micelles may be a means of stabilizing the protein structure.

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