Role of Prenylation in the Interaction of the a-Factor Mating Pheromone with Phospholipid Bilayers†

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ABSTRACT: We have studied the interaction between phospholipids and a-factor (YIIKGVFWDPAC-[Farn]OMe), *S*-alkylated forms of a-factor with the farnesyl group substituted by methyl, hexadecanyl, or benzyl groups, and truncated forms of this lipopeptide. Circular dichroism studies suggest that, despite its lack of farnesylation, *S*-methyl-a-factor is incorporated into vesicles of dimyristoylphosphatidylcholine in a conformation similar to that which a-factor adopts in this membrane. However, studies of the intrinsic fluorescence of the Trp residues of these peptides indicate that this residue is more deeply imbedded into the bilayer in the case of the farnesylated peptide. The a-factor is more effective in raising the bilayer to the hexagonal phase transition temperature of dielaidoylphosphatidylethanolamine than is the *S*-methyl-a-factor. This bilayer-stabilizing ability is also reflected in a-factor inhibiting leakage from vesicles of *N*-methyldioleoylphosphatidylethanolamine. Studies on a-factor analogs permit the conclusion that the bilayer-stabilizing effect of a-factor is not solely a consequence of its greater partitioning into the membrane but is also a consequence of the degree of penetration into the bilayer and the specific conformation of the peptide at the membrane interface. These results indicate that the farnesyl group alone, in the absence of cellular factors, bestows a particular physical interaction with membranes.

Isoprenylation has been recently recognized as a common post-translational modification of proteins [for reviews, see Clarke (1992) and Schafer and Rine (1992)]. This modification has been shown to have functional significance for the biological activity of many polypeptides, including fungal pheromones such as the a-factor of *Saccharomyces cerevisiae* (Anderegg et al., 1988), small GTP-binding proteins such as Ras, Rho, Rap, and Rab/Ypt (Chardin, 1988), components of the heterotrimeric G-proteins such as bovine transducin (Lai et al., 1990), and nuclear lamins (Wolda & Glomset, 1988). Indeed mutant Ras proteins do not manifest oncogenicity in mammalian cells unless they are farnesylated (Der & Cox, 1991; Kato et al., 1992). It has recently been shown that farnesylation of Ras2 is necessary to obtain optimal binding to adenylyl cyclase but is not sufficient for maximal partitioning of Ras2 into membranes (Kuroda et al., 1993). Although there is considerable evidence that many functionally important proteins are isoprenylated and that isoprenylation may be required for their function or for their subcellular localization to the nuclear, organelle, or cytoplasmic membrane, there has not been a systematic study of the consequences of this modification on peptide–lipid interactions.

Three potential models for a mechanism(s) of targeting have been suggested recently by Schafer and Rine (1992), who add that additional cellular factors could be involved in these localizations. They propose that prenylated proteins might be directed to membranes by specific membrane-bound receptors, by nonspecific or specific interaction between the prenyl group and membrane lipids, or by a secondary modification subsequent to prenylation which is the actual membrane localization signal. We believe that an understanding of the biophysical nature of the interaction of prenylated peptides with model membranes will help to resolve the underlying molecular properties involved in the targeting of prenylated peptides in cells.

We have chosen to study the interaction between lipids and the yeast mating pheromone, a-factor, and its analogs. This lipopeptide with the amino acid sequence YIIKGVFWDPAC is farnesylated on the side-chain thiol group, and the terminal carboxyl group is in the form of a methyl ester (Figure 1). It is particularly attractive to study a-factor because of its easily assayed biological activity and because its relatively small size allows for the synthesis of analogs.

Experimental approaches to investigate lipopeptide–membrane interactions include measuring the tendency of bilayer membranes to be converted to the inverted hexagonal phase (H_{II})[1] in the presence of prenylated peptides. This membrane property is associated with certain membrane functions, such as membrane fusion and the activation of protein kinase C (Epand, 1992). The relative stabilities of the bilayer and H_{II} phases can be evaluated by measuring the effects of additives on the bilayer to H_{II} phase transition temperature (T_H). The effect of lipopeptides on leakage from liposomes composed of stable bilayers can also be determined. In this study, the above procedures, along with circular dichroism and fluorescence measurements, were applied to evaluate the role of farnesylation in the conformation of a-factor in the presence

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[1] Abbreviations: TLC, thin-layer chromatography; DSC, differential scanning calorimetry; CD, circular dichroism; *N*-methyl-DOPE, *N*-methyl-1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; DEPE, 1,2-dielaidoyl-*sn*-glycero-3-phosphoethanolamine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; lyso-PC, 1-palmitoyllysophosphatidylcholine; LUV, large unilamellar vesicle; DPX, *p*-xylylenebis(pyridinium bromide); ANTS, aminonaphthalene-3,6,8-trisulfonic acid; H_{II}, inverted hexagonal phase; T_H, lamellar to H_{II} phase transition temperature.

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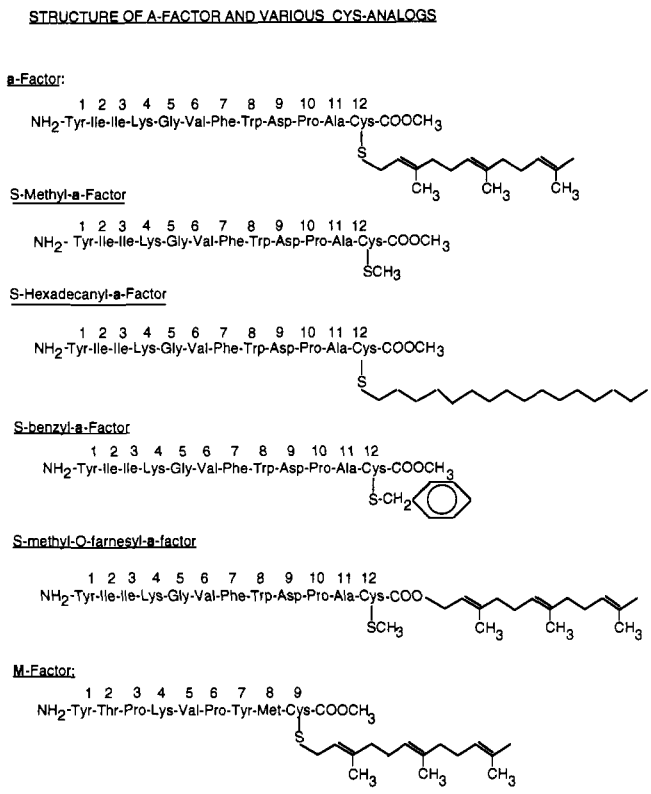

FIGURE 1: Structure of a-factor and various analogs.

of amphiphiles, in influencing its depth of penetration in membranes and micelles, and in stabilizing lipid vesicles.

EXPERIMENTAL PROCEDURES

Materials

Details on the synthesis of the a-factor, the *S*-benzyl-a-factor, the *S*-hexadecanyl-a-factor, and the *S*-methyl-a-factor have been previously published (Xue et al., 1991). These peptides were prepared by a combination of solution-phase and solid-phase synthesis (Xue et al., 1989, 1991). The other peptides used in this investigation were prepared using identical strategies. Details will be published elsewhere. All peptides were purified to over 98% homogeneity as judged using acetonitrile/water/trifluoroacetic acid gradients and methanol/water/trifluoroacetic acid gradients on reversed-phase HPLC and were characterized by amino acid analysis and fast atomic bombardment (FAB) mass spectrometry. Biological assays for the a-factor, the *S*-benzyl-a-factor, the *S*-hexadecanyl-a-factor, and the *S*-methyl-a-factor, and the farnesol ester containing analogs have been reported (Marcus et al., 1991; Xue et al., 1992). An evaluation of the effect of truncation on biological activity of the a-factor is in progress and will be reported elsewhere.

Phospholipids used in this work were obtained from Avanti Polar Lipids (Birmingham, AL). They were shown to be over 98% pure. Fluorescent probes were purchased from Molecular Probes (Eugene, OR).

Methods

Circular Dichroism. The circular dichroism (CD) spectra of the peptides were recorded on an AVIV model 61 DS solid-state CD instrument (AVIV Associates, Lakewood, NJ). The instrument was interfaced with a computer, which was used for all mathematical calculations. A 1-mm sample cell, maintained at 25 °C with a thermostated cell holder, was used for all spectral studies. Peptide concentrations in solution, prior to the addition of lipid, were determined from the ultraviolet absorption at 280 nm.

Fluorescence Spectra. Fluorescence emission spectra were recorded on an SLM-Aminco Series 2 luminescence spectrometer.

Differential Scanning Calorimetry (DSC). Lipid films were made from DEPE dissolved in chloroform/methanol (2:1, v/v) to which varying quantities of peptide dissolved in methanol were added. The peptide concentration was estimated by weight. After solvent evaporation with nitrogen, final traces of solvent were removed in a vacuum chamber for 90 min. The lipid films were suspended in 20 mM PIPES, 1 mM EDTA, and 150 mM NaCl with 0.002% NaN_3, pH 7.40, by vortexing at 45 °C for 30 s. The final lipid concentration was 5 mg/mL. The lipid suspensions were degassed under vacuum before being loaded into an MC-2 high-sensitivity scanning calorimeter (Microcal Co., Amherst, MA). A heating scan rate of 39 K/h was employed. The bilayer to hexagonal phase transition was fitted to a single van't Hoff component and the transition temperature reported as that for the fitted curve.

Leakage Assay. The ANTS–DPX leakage assays were carried out as described by Ellens et al. (1985). Large unilamellar vesicles of *N*-methyl-DOPE were prepared at pH 9.5 by extrusion. The high pH is required to form stable liposomes with this lipid. A lipid suspension was made to undergo five freeze–thaw cycles and then passed 10 times through a Lipex (Vancouver, BC) extruder using 0.1-μm Nucleopore filters. Vesicles were passed through a Sephadex G-75 column and contained 12.5 mM ANTS, 45 mM DPX, and 22.5 mM NaCl entrapped at pH 9.5. Fluorescence intensity was monitored with an excitation wavelength of 380 nm and an emission wavelength of 510 nm. All assays were carried out in a total volume of 1 mL. The final lipid concentration was 0.4 μmol/mL. Leakage was initiated by lowering the pH from 9.5 to 4.5 with 25 μL of 2 M sodium acetate/acetic acid buffer. At the lower pH, vesicles of *N*-methyl-DOPE become unstable and begin to leak their internal contents. Leakage and the effects of the lipopeptides on this leakage were measured by dequenching of fluorescence due to dilution and the dissociation of the ANTS-DPX complex.

Membrane Partitioning Assay. To 5 mg of *N*-methyl-DOPE prepared as a dried film on the walls of a test tube was added 1.0 mL of peptide solution. The peptides were dissolved with difficulty by briefly sonicating, vortexing, and warming into the pH 7.4 Pipes buffer used for DSC. The peptide concentration was determined by absorbance at 280 nm. Lipid-bound peptide was separated from peptide in solution by centrifugation at 100000*g* for 20 min at 25 °C in a TLA-100.2 rotor of a Beckman TL-100 centrifuge. Controls were done to ensure that the concentration of peptide in solution in the absence of lipids did not change by this procedure. The peptide concentrations were about 0.2 mM, giving a lipid/peptide molar ratio of about 30.

RESULTS

It has been shown by two-dimensional NMR methods that the a-factor in DMSO is largely structureless and that *S*-prenylation of the Cys^{12} does not induce folding (Goundarides et al., 1991). Circular dichroism (CD) is a useful method for the conformational analysis of peptides and proteins and is often used to obtain an estimate of the secondary structure of proteins. The CD profiles of both a-factor and *S*-methyl-a-factor in the presence of vesicles of DMPC are

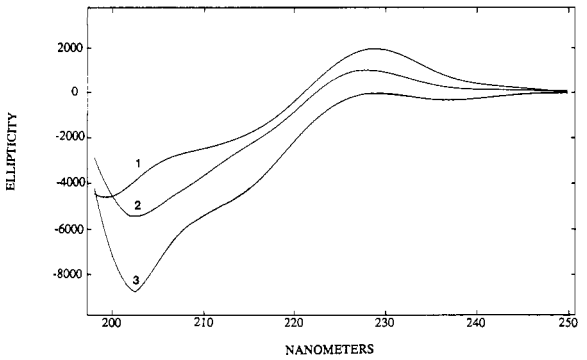

FIGURE 2: Circular dichroism spectra of a-factor and *S*-methyl-a-factor. Ellipticity values are given as mean residue ellipticity in units of deg·cm^2 dmol^{-1}. The peptide concentration was 250 μM in 10 mM sodium phosphate buffer, pH 7.4. DMPC, when present, is at a 6.5-fold molar excess over peptide. Temperature: 25 °C. Curve 1, a-factor with DMPC; curve 2, *S*-methyl-a-factor with DMPC; curve 3, *S*-methyl-a-factor without lipid.

Table I: Comparison of Fluorescence Emission Maxima of a-Factor and of *S*-Methyl-a-factor[a]

condition	λ_{em} (nm)	
	a-factor	*S*-methyl-a-factor
buffer	*b*	352
methanol	343	343
lyso-PC	343	341
DMPC	329	349

[a] Peptides were dissolved in methanol or in 10 mM sodium phosphate buffer, pH 7.4. Temperature: 25 °C. Excitation wavelengths of 280 or 295 nm gave similar results as did changing the molar ratio of amphiphile to peptide from 20 to 40. Peptide concentrations are about 300 μM. [b] Too insoluble to measure.

not typical of peptides in either an α-helical or β-sheet conformation (Figure 2). The mean residue ellipticities are very low, and the peak associated with the $\pi \rightarrow \pi^*$ transition appears at about 198 nm in the a-factor and 202 nm in the *S*-methyl-a-factor. Although the a-factor exhibits a minor shoulder in the n $\rightarrow \pi^*$ region, the curves for both peptides are quite similar and suggestive of primarily disordered peptides. Interestingly, the *S*-methyl-a-factor in aqueous buffer exhibits a CD curve suggestive of more structure. Although the ellipticities are still quite low, the change in the pattern would be consistent with increased β-structure for this peptide. The a-factor was not sufficiently soluble in water to obtain the CD spectrum in the absence of phospholipid. Similar results were found in the presence of micelles of lyso-PC or when dissolved in methanol (data not shown). Estimates of the percentages of the various secondary structures of such short peptides are prone to inaccuracy, and quantitative conclusions from the CD studies are not warranted. However, the comparison of the farnesylated and the *S*-methylated peptide suggests that even the latter partitions into the lipid.

To further assess the lipid penetration of the a-factor and its analogs, the fluorescence emission spectrum of these peptides was measured in different solvents. Assuming that relaxation of the surrounding dipoles is more rapid than singlet excitation decay, then the lower the emission wavelength is of the Trp residue, the less polar is the solvent environment of this side chain. As expected, both a-factor and *S*-methyl-a-factor have similar fluorescent emission spectra in methanol (Table I). The lower emission wavelength of the *S*-methyl-a-factor in methanol compared with that in water is indicative of the change in solvent polarity. Both peptides also behave similarly in the presence of lyso-PC and appear to be in an environment with a polarity similar to that of methanol. In the presence of sonicated vesicles of DMPC, however, there is a marked difference, with the a-factor appearing to insert more deeply into the lipid bilayer than the *S*-methyl-a-factor. This difference is not a result of differences in inherent fluorescence emission properties of the two peptides since the peptides show the same emission maximum in a solution of methanol, where the Trp is likely to be fully exposed to the solvent. Comparison between lyso-PC and DMPC is interesting. It suggests that a-factor is able to embed into a more hydrophobic environment in a bilayer than in a micelle, while the opposite is the case for the *S*-methyl-a-factor.

Table II: Effect of a-Factor and Related Compounds on the T_H of DEPE

substance	slope (deg/mole fraction additive)
a-factor	217 ± 14
[D-Cys^{12}]-a-factor	211 ± 24
[D-*S*-methyl-*O*-farnesyl-Cys^{12}]-a-factor	198 ± 14
trans,trans-farnesol	−188 ± 54
trans,trans-farnesol acetate	−133 ± 27
hexadecanol	−145 ± 8
S-hexadecanyl-a-factor	106 ± 19
benzyl alcohol	49 ± 32
S-benzyl-a-factor	112 ± 50
S-methyl-a-factor	31 ± 16
S-methyl-a-factor + farnesol[a]	−194 ± 10
des-Tyr^1-a-factor	264 ± 63
des-Tyr^1,Ile^2-a-factor	146 ± 40
des-Tyr^1,Ile^2,Ile^3-a-factor	269 ± 13
des-Tyr^1,Ile^2,Ile^3,Lys^4-a-factor	164 ± 10
YTPKVPYMC (farnesyl) OMe (M-factor)	30 ± 38

[a] Equimolar amounts of these materials were used.

The shift of the bilayer to hexagonal phase transition temperature (T_H) of dielaidoylphosphatidylethanolamine (DEPE) with the addition of a membrane additive is a measure of how this additive affects the relative stability of the bilayer vs nonlamellar inverted phases. This information has proven to be of value in predicting the effect of these additives on certain membrane functional properties (Epand, 1992). In order to compare various a-factor derivatives, we measured the slope of a plot of T_H versus the mole fraction of added lipopeptide (Table II). Positive slopes indicate bilayer stabilizers (relative to the H_{II} phase) whereas negative slopes indicate compounds that cause a relative stabilization of the hexagonal phase. In the case of a-factor, the shift in T_H is proportional to the mole fraction of peptide, suggesting that the peptide and lipid are largely miscible (Figure 3). We have studied several modified forms of a-factor which have the same chemical groups and therefore are likely to have the same hydrophobicities, but with altered stereochemical arrangements. Inversion of the Cys^{12} αCH or switching the position of the methyl and farnesyl groups on the D-Cys do not alter the effect of these peptides on the T_H of DEPE (Table II, Figure 3). However, [*S*-methyl-*O*-farnesyl-Cys^{12}]-a-factor does exhibit altered behavior, having marked bilayer stabilizing properties at low mole fractions but with the effect rapidly saturating (Figure 3). The fluorescence emission maximum from this analog in the presence of *N*-methyl-DOPE is intermediate between that of a-factor and *S*-methyl-a-factor (data not shown). Although we do not have an explanation for the different behavior of this analog, the results do demonstrate the sensitivity of the bilayer-stabilizing effect to the steric arrangement of groups in the lipopeptide. The altered feature which most likely accounts for the modified behavior of the [*S*-methyl-*O*-farnesyl-Cys^{12}]-a-factor is the orientation

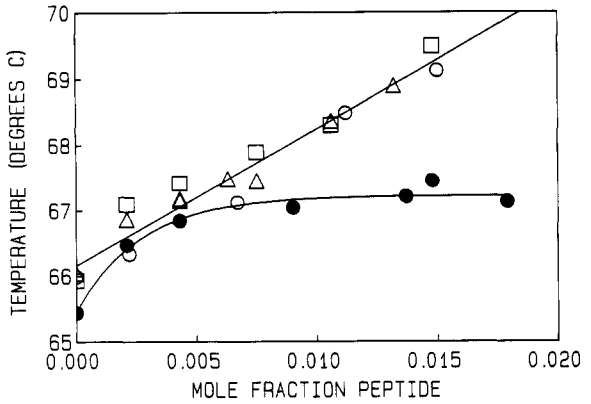

FIGURE 3: Effect of a-factor and analogs on the T_H of DEPE. a-factor, ○; [D-Cys[12]]-a-factor, □; [D-*S*-methyl-*O*-farnesyl-Cys[12]]-a-factor, △; [L-*S*-methyl-*O*-farnesyl-Cys[12]]-a-factor, ●.

of the peptide moiety of a-factor with respect to the bilayer surface.

We have examined the influence of the nature of the hydrocarbon group attached to the Cys[12] sulfur on the bilayer-stabilizing tendency of the peptide. The natural form of a-factor is farnesylated at this position. The farnesyl group in isolation is a hexagonal phase promoter (Table II). Despite this, when the farnesyl group is attached to the peptide, the resulting a-factor becomes a good bilayer stabilizer. Farnesylation appears to orient the lipopeptide in a manner which leads to greater bilayer stabilization than is observed with either the farnesyl group or the peptide alone.

Indeed, when equimolar amounts of *S*-methyl-a-factor and farnesol were added to DEPE, the T_H was lowered by an amount similar to that found with farnesol alone (Table II). Similar results were obtained with this peptide mixed with farnesol acetate. Thus, the types of membrane interactions exhibited by a-factor are a consequence of the covalent linkage of the peptide and lipid moieties and cannot be simulated by a simple mixture of these two components. The change as a result of farnesylation is not simply a consequence of increased hydrophobicity and/or membrane partitioning of the peptide. This is clearly demonstrated by comparing *S*-hexadecanyl-a-factor with a-factor. Both the hexadecanol and farnesol would be expected to have similar hydrophobicities (Black, 1992) and therefore both would affect partitioning of the lipopeptide into a membrane to a similar extent. If anything, the hexadecanyl peptide may be somewhat more hydrophobic as indicated by its greater retention on reversed-phase HPLC (data not shown). Both farnesol and hexadecanol have similar effects on T_H, yet farnesylation leads to a lipopeptide (a-factor) which is a much better bilayer stabilizer than is *S*-hexadecanyl-a-factor (Table II). Even a benzyl group, which as illustrated by benzyl alcohol is a bilayer stabilizer by itself, is not able to shift T_H as much as farnesylation does.

To evaluate the importance of the peptide structure on the interaction of farnesylated peptides with lipids, we measured the influence of truncated forms of a-factor on T_H. The des-Tyr[1]-a-factor, IIKGVFWDPAC (farnesyl) OMe, is a potent bilayer stabilizer (Table II). As additional residues are removed from the N-terminus, T_H decreases and increases in a periodic manner (Table II). Nevertheless, all truncated peptides remain effective bilayer stabilizers for DEPE, and KGVFWDPAC(farnesyl)OMe, which lacks the three hydrophobic N-terminal residues of a-factor, is a more potent stabilizer than the parent lipopeptide. The more hydrophilic lipopeptide, YTPKVPYMC(Farnseyl)OMe (the M-factor from *Schizosaccharomyces pombe*), is a marginal bilayer

Table III: Fluorescence Emission Maxima of a-Factor and Analogs in the Presence of *N*-Methyl-DOPE at a Lipid to Peptide Ratio of 18[a]

peptide	λ_{em} (nm)
a-factor	327
des-Tyr[1]-a-factor	329
des-Tyr[1],Ile[2]-a-factor	335
des-Tyr[1],Ile[2],Ile[3]-a-factor	341
des-Tyr[1],Ile[2],Ile[3],Lys[4]-a-factor	342
S-methyl-a-factor	349

[a] Conditions: temperature, 25 °C; 10 mM sodium phosphate buffer, pH 7.4; excitation at 280 nm.

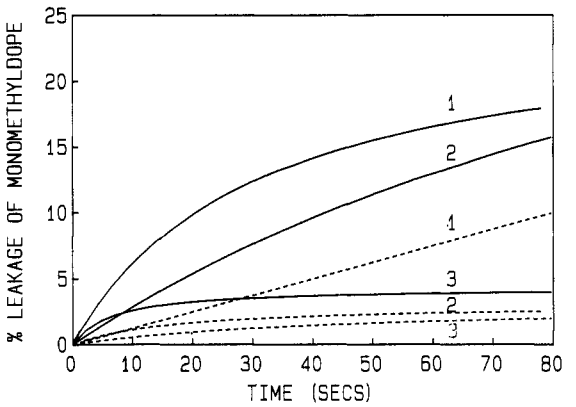

FIGURE 4: Lipopeptide-induced leakage from vesicles of *N*-methyl-DOPE at 28 °C (dashed lines) or 48 °C (solid lines). Lipid concentration was 370 μM with a lipid to peptide ratio of 7. Curve 1, *S*-methyl-a-factor; curve 2, control vesicles, no peptide added; curve 3, a-factor.

stabilizer, but covalent linkage of the farnesyl moiety to this peptide still reverses the H_{II}-promoting tendencies of farnesol.

The insertion of the truncated analogs into bilayers of phospholipid vesicles was tested using LUVs composed of *N*-methyldioleoylphosphatidylethanolamine (*N*-methyl-DOPE). These vesicles were also used for leakage studies since LUVs can be formed more readily from this lipid than from DEPE. As the peptide is shortened or if the farnesyl group is replaced by methyl, the λ_{em} shifts toward the red (Table III). Interestingly, des-Tyr[1],Ile[2],Ile[3]-a-factor and des-Tyr[1],Ile[2],-Ile[3],Lys[4]-a-factor have almost identical λ_{em}. For most of the truncated a-factor peptides, there is a reasonable correlation between the length of the peptide and the degree to which the Trp residue appears to be buried in the membrane. However, the λ_{em} of a peptide is not a good indicator of the influence of these farnesylated peptides on T_H.

The interaction of farnesylated peptides with bilayers was also assessed by determining vesicle leakage in the presence of different analogs. Leakage from LUVs of *N*-methyl-DOPE was promoted by *S*-methyl-a-factor but inhibited by a-factor (Figure 4). The truncated analogs of a-factor either inhibited leakage like a-factor itself, or slightly stimulated leakage, depending on the lipid/peptide ratio (data not shown). None of the truncated analogs stimulated leakage as much as the *S*-methyl-a-factor. This is in agreement with the greater effects of the truncated analogs in raising T_H of DEPE. In general, substances which raise T_H inhibit leakage from vesicles which have a marked tendency to convert to the H_{II} phase. The converse is also true for hexagonal phase promoters, although this does not mean that the liposomes have to form H_{II} phases in order to leak. Changes in bilayer properties, making them more or less prone to form nonbilayer phases, will determine the rate of leakage [e.g., see Cheetham et al. (1990)] without nonbilayer phases necessarily having to form.

The *S*-methyl-a-factor has minimal influence on the T_H of DEPE, suggesting that it neither stabilizes nor destabilizes the bilayer. Nevertheless it is clear that this peptide does interact with liposomes (*vida infra*). Since the *S*-methyl-a-factor interacts with bilayers but does not stabilize them, it is not surprising that the peptide causes increased vesicle leakage. Moreover, although different lipids were used in the bilayer stabilization and leakage studies, the fact that a-factor inhibits leakage whereas *S*-methyl-a-factor promotes leakage is compatible with their respective influences on T_H.

The fact that *S*-methyl-a-factor has effects on T_H, as well as on vesicle leakage and fusion, indicates that this peptide binds to lipid. We determined how much of this peptide partitions into *N*-methyl-DOPE by separating free from bound peptide by centrifugation. This could not be done with the a-factor because of its poor solubility in water. However, because of the addition of the farnesyl chain, the a-factor will partition into the membrane to a greater extent than the *S*-methyl-a-factor. At a 50:1 lipid to peptide ratio, 17% of the *S*-methyl-a-factor partitions into the membrane at room temperature. Thus, despite the fact that only a fraction of the *S*-methyl-a-factor partitions into lipid, it is still potent in promoting leakage (Figure 4). However, the weak effect of *S*-methyl-a-factor on lipid polymorphism (Table II) can, at least in part, be due to incomplete partitioning of this analog into the membrane. The differences in the membrane partitioning of *S*-methyl-a-factor and a-factor cannot account for the observation that these two peptides have opposite effects on vesicle leakage. These opposite effects on leakage are explained, as stated above, by their respective different influence on T_H.

DISCUSSION

Covalent attachment of a lipid moiety to a peptide or protein may have a variety of consequences. It is likely to promote the partitioning of peptides from an aqueous to a membranous environment. However, neither farnesylation nor geranylgeranylation is sufficient to cause membrane association of mutant forms of the γ-subunit of an inhibitory G-protein (Butrynski et al., 1992). Attachment of the lipid moiety may alter the conformation of the membrane-bound peptide (Macquaire et al., 1992) or its location with respect to the membrane/water interface.

Our studies with a-factor and its analogs provide the first direct physical evidence that farnesylation of the Cys sulfur atom results in a lipopeptide that affects membrane properties in a manner which is different from either that of the unmodified peptide moiety or the free farnesyl group. This is demonstrated by a marked blue-shift in the fluorescence emission of the Trp, by the large shift in T_H of membrane lipids caused by the farnesylated peptide, and by the opposite effects of the *S*-methyl-a-factor and the unmodified a-factor on vesicle leakage. Farnesylation apparently results in a change in the way that a peptide alters membrane properties, which may indicate that farnesylation modifies the orientation of this peptide at the membrane surface. This is likely to have consequences not only on membrane properties but also on which groups or protein sites in a biological membrane will have access to the lipopeptide. For example, farnesylation is necessary for Ras2 proteins of yeast to bind with maximum affinity to adenylyl cyclase (Kuroda et al., 1993).

The interaction of hydrophobic peptides with lipids is influenced by various properties of the peptide such as amino acid sequence (Surewicz & Epand, 1985), length (McLean et al., 1991), and net change (Jacobs & White, 1986). In our studies, we determined the interaction of a series of truncated forms of a-factor with lipid. As the length of the peptide was shortened, the net charge remained the same until removal of the Lys^4, but the sequence and hydrophobicity varied significantly. For this reason, we are not able to exactly dissect the contributions of length, sequence, and charge on lipid interaction. Nevertheless, our results indicate that all of the truncated forms effect T_H and that the tryptophan residue penetrates less deeply into the membrane as the peptide is shortened. These results must be considered along with our findings that truncated a-factors have lower biological activity than the full-length pheromone (Caldwell, personal communication).

The results reported herein establish that the farnesyl group results in a very specific association of the a-factor with membranes. Whether this association is sufficient to manifest the biological activity of the pheromone has not been determined. It is clear, however, that the nature of the membrane association and the penetration of the peptide into the hydrophobic portion of the bilayer change with the alkyl group and with the topology of the hydrophobic groups at the carboxyl terminus of the a-factor. Thus, the L-[*S*-methyl-*O*-farnesyl]-a-factor affects the transition temperature differently than the other isomers in this series (Figure 3). All of these molecules are identical except for the stereochemical relationships of the methyl and farnesyl moieties. Furthermore, although removal of the farnesyl group from a-factor results in a 10^2–10^3-fold decrease in biological potency, replacement of this moiety with the highly hydrophobic hexadecanyl substituent does not result in a highly active analog (Marcus et al., 1991). We suggest, therefore, that the interaction of the farnesyl side chain with the bilayer results in a presentation of the pheromone to the receptor, which is an important determinant of activity.

With respect to the model of Schafer and Rine (1992), direct interactions between membrane lipid and farnesylated peptide appear to result in major physical changes in both peptide and membrane. However, cellular factors may still be required for proper secretion of the pheromone from the producing cell (Mata cell) and other or similar factors may be needed for the correct interaction of the pheromone with its receptor on the target cell (Matα cell). Furthermore, the situation of a small peptide pheromone may not be analogous to the association of prenylated proteins to their subcellular locations.

In conclusion, our findings indicate that the farnesyl group imparts special properties to the a-factor pheromone which lead to a particular association and/or orientation in membranes. It will be interesting to ascertain whether this association is necessary for a productive biological interaction with its secretory protein (encoded by the *STE6* gene) or its receptor (encoded by the *STE3* gene). Such investigations await isolation of these gene products and their reconstitution in model membranes.

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