Fluorescence Analysis of Tryptophan-Containing Variants of the LamB Signal Sequence upon Insertion into a Lipid Bilayer†

C. James McKnight,‡ Maria Rafalski,§ and Lila M. Gierasch*

Departments of Pharmacology and Biochemistry, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, Texas 75235

Received December 28, 1990; Revised Manuscript Received March 15, 1991

ABSTRACT: To investigate the interaction of the LamB signal sequence with lipid bilayers, we have synthesized three tryptophan-containing analogues of the wild-type signal peptide. The tryptophan residues were used as intrinsic fluorescent probes of the N-terminal (position 5), central (position 18), and C-terminal (position 24) regions of the 25-residue peptide. The tryptophan substitutions did not significantly alter the physical properties of the wild-type signal peptide. In the presence of lipid vesicles which mimic the composition of the *Escherichia coli* inner membrane, the peptides adopt α-helical structure, and the tryptophan fluorescence emission maximum is shifted to shorter wavelength, indicating that the peptides insert into the acyl chain region of the lipid bilayer. Fluorescence quenching by soluble, aqueous-phase (I^-), and membrane-resident (nitroxide-labeled lipids) quenchers was used to locate the tryptophans in each peptide within the bilayer. The C-terminus was interfacial while the central region of the signal sequence was deeply buried within the acyl chain region of the bilayer. The tryptophan at position 5 was buried but less deeply than the tryptophan at position 18. This topology is consistent with either a looped or a transmembrane orientation of signal peptide. However, either structure must accommodate the high helical content of the peptides in vesicles. These results indicate that the LamB signal sequence spontaneously inserts into the acyl chain region of lipid membranes in the absence of any of the proteins involved in protein secretion.

The transient, N-terminal signal sequence is the most general requirement for the export of secretory proteins from the cytosol [for reviews, see Gierasch (1989), Verner and Schatz (1988), and Wickner (1988)]. The signal sequence appears to function in several roles during export including (i) recognition by targeting factors [e.g., signal recognition particle (SRP)[1] in eukaryotes (Walter & Blobel, 1983) and possibly SecA (Schmidt et al., 1988) and SecB (Collier et al., 1988; Watanabe & Blobel, 1989) in prokaryotes], (ii) interaction with membrane proteins [e.g., the signal sequence receptor (Hartmann et al., 1989) in eukaryotes and SecY (Emr et al., 1981; Ito, 1984) in prokaryotes], and (iii) recognition by the specific signal peptidases responsible for cleavage of the signal sequence from the mature protein. For the majority of transported proteins, an energy-utilizing, proteinaceous machinery is required for export (Chen & Tai, 1985; Connolly & Gilmore, 1989). The nature of the site of translocation is not clear, and whether the protein trasverses the membrane through a water-filled channel or is exposed to the lipid phase of the membrane is unknown.

Another proposed function of the signal sequence, based on its highly hydrophobic nature, is direct insertion into the lipid phase of the membrane (von Heijne & Blomberg, 1979; Wickner, 1980; Engelman & Steitz, 1981). The insertion of the signal sequence into the membrane would lead to local membrane perturbations (Killian et al., 1990a), which could facilitate transport directly through the lipid phase. Indeed, the small coat protein of the M13 virus is translocated into lipid vesicles in the absence of any proteins (Geller & Wickner, 1985). Alternatively, the signal sequence might insert into the lipid phase and promote assembly of the export machinery.

Studies of synthetic peptides corresponding to signal sequences suggest that they insert spontaneously into lipid bilayers. Synthetic signal peptides have been shown to insert into lipid monolayers (Cornell et al., 1989; McKnight et al., 1989; Batenburg et al., 1988a). Changes in the conformations of signal peptides in the presence of phospholipid vesicles also suggest an interaction of the signal peptides with lipid bilayers (McKnight et al., 1989; Batenburg et al., 1988b; Shinnar & Kaiser, 1984). Recently, Killian et al. (1990b) used tryptophan fluorescence to show that the hydrophobic region of the PhoE signal peptide was sequestered within the bilayer of lipid vesicles.

To examine the topology of the LamB signal peptide in a bilayer environment, three peptides that contain tryptophan residues in different regions of the wild-type LamB signal sequence were designed. The tryptophan residues were used as intrinsic fluorescent probes to report the local environment of the N-terminus, the central segment, or the C-terminus of the peptide. Steady-state fluorescence emission spectroscopy and fluorescence quenching were used to localize the tryptophan residues of the peptides in relation in the lipid bilayer. Our results indicate that the LamB signal sequence inserts deeply into the acyl chain region of lipid bilayers, either in a

† Supported by NSF Grant DCB-8896144 and the Robert A. Welch Foundation.

* Author to whom correspondence should be addressed at the Department of Pharmacology, University of Texas Southwestern Medical Center.

‡ Present address: Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142.

§ Present address: DuPont Merck Pharmaceutical Co., P.O. Box 80328, Wilmington, DE 19880-0328.

[1] Abbreviations: CD, circular dichroism; HPLC, high-pressure liquid chromatography; LUV, large unilamellar vesicle; NMR, nuclear magnetic resonance; POPE, 1-palmitoyl-2-oleoylphosphatidylethanolamine; POPG, 1-palmitoyl-2-oleoylphosphatidylglycerol; PSPC, 1-palmitoyl-2-stearoylphosphatidylcholine; SRP, signal recognition particle; SUV, small unilamellar vesicle; Tris, tris(hydroxymethyl)aminomethane.

0006-2960/91/0430-6241$02.50/0

looped or in a transmembrane orientation.

Materials and Methods

1-Palmitoyl-2-oleoylphosphatidylethanolamine (POPE), 1-palmitoyl-2-oleoylphosphatidylglycerol (POPG), 1-palmitoyl-2-stearoylphosphatidylcholine (PSPC), and nitroxide-labeled lipids were purchased from Avanti Polar Lipids. Lipid purity was routinely checked by thin-layer chromatography on silica plates run in chloroform/methanol/water (65/35/4) and developed with I_2 vapor.

Synthesis and Purification of Peptides. Peptides were synthesized via standard peptide synthesis methodology using N-terminal, *t*-Boc-protected amino acids (Erickson & Merrifield, 1976; Barany & Merrifield, 1979). Peptides were deprotected and cleaved from the resin by treatment with anhydrous HF. Peptides were purified by reverse-phase high-performance liquid chromatography (HPLC) on Vydac C_4, C_8, and/or phenyl columns eluted with acetonitrile containing 0.1% trifluoroacetic acid. Amino acid content and peptide concentrations were determined by quantitative amino acid analysis. The sequences of the purified peptides were confirmed by peptide sequencing.

Preparation of Vesicles. Lipids (10–25 mg) of the desired composition were mixed in chloroform and then dried in vacuo for at least 2 h. The lipid mixtures were hydrated with 1.0 mL of 5 mM tris(hydroxymethyl)aminomethane (Tris), pH 7.3. For the preparation of small unilamellar vesicles (SUVs), the lipid suspensions were degassed with N_2 and sonicated in a bath sonicator (Laboratory Supplies Co.) until the suspension was clear (approximately 1 h). For large unilamellar vesicles (LUVs), the lipid suspensions were then freeze–thawed 5 times between liquid N_2 and warm water to facilitate the mixing of compartments, followed by extrusion 10 times through two, 0.1-μm pore-size polycarbonate filters (Nucleopore) with nitrogen pressure in an extrusion device (Lipex Biomembranes). This procedure yields LUVs with an average diameter of 90 nm (Mayer et al., 1983). Lipid concentrations were determined by inorganic phosphate analysis (Fiske & SubbaRow, 1925) of ashed phospholipid samples (Ames, 1966).

Circular Dichroism (CD) Measurements. CD measurements were performed on an AVIV 60DS spectrophotometer equipped with a Hewlett Packard 89100A temperature controller. SUVs of 65% POPE/35% POPG were used to minimize light-scattering artifacts. Peptide concentrations were 5 μM, and the SUV lipid concentration was 1 mM. Spectra were obtained at 25 °C in a 1-mm quartz cell (Helma). Spectra were base-line-corrected, converted to mean residue ellipticity, and smoothed by using a polynomial fitting function (Aviv Associates, Inc.).

Fluorescence Measurements. Fluorescence measurements were obtained on a steady-state, photon counting spectrofluorometer (Model Greg PC from ISS Inc.) operated in the ratio mode. Emission and excitation slit widths were 8 or 16 nm. All spectra were measured by using a 1-cm-square quartz fluorescence cuvette thermostated at 25 °C and continuously stirred. Emission spectra were measured every 1 or 2 nm with an averaging time of 3–5 s/nm. The excitation wavelength for all spectra was 280 nm. To correct for excitation light scattered by LUVs, spectra of samples containing more than 0.1 mM lipid were base-line-corrected by subtraction of spectra of the same concentration of LUVs without fluorophore. Emission maxima were determined by visual examination of raw or base-line-corrected spectra.

Iodide Quenching Experiments. All solutions used in the iodide quenching experiments contained 0.1 mM $Na_2S_2O_3$ as a reductant to prevent the formation of significant amounts of I^{3-}. Samples were made in advance of the experiment with varying amounts of NaI, and the salt concentration was maintained at 0.2 M with NaCl. SUVs were prepared as described above in 0.2 M NaCl, 10 mM Tris, pH 7.3, and 0.1 mM $Na_2S_2O_3$. I_0/I values were calculated from base-line-corrected spectra by the fluorescence intensities at the tryptophan emission maxima of samples with and without iodide.

Spin-Labeled LUV Quenching Experiments. Spin-labeled LUVs were prepared by extrusion in 5 mM Tris, pH 7.3. Lipid compositions were 55% POPE/35% POPG/10% 1-palmitoyl-2-(5-doxylstearoyl)phosphatidylcholine (5-doxyl-PC), 1-palmitoyl-2-(12-doxylstearoyl)phosphatidylcholine (12-doxyl-PC), 1-palmitoyl-2-(16-doxylstearoyl)phosphatidylcholine (16-doxyl-PC), or PSPC for control vesicles. I_0/I values were calculated from base-line-corrected spectra by the fluorescence intensities at the tryptophan emission maxima of samples without and with spin-label.

Table I: Sequences of Tryptophan-Containing LamB Signal Sequences[a]

	1	5	10	15	20	25
WT	M M I T L R K L P L A V A V A A G V M S A-Q A M A /					
5W	M M I T W R K L P L A V A V A A G V M S A Q A M A /					
18W	M M I T L R K L P L A V A V A A G W M S A Q A M A /					
24W	M M I T L R K L P L A V A V A A G V M S A Q A W A /					

[a] Numbers indicate amino acid position from the N-terminus, slants indicate the signal peptidase cleavage site. Underlined characters indicate residues differing from the wild-type sequence.

Results

Design of Tryptophan-Containing Peptides. Three peptides were designed to take advantage of the properties of tryptophan fluorescence to aid in elucidating the signal peptide–lipid bilayer interaction (Table I). Each peptide contains a tryptophan residue in a different part of the wild-type LamB signal sequence to report on the environment of that region. In each substitution, a bulky, hydrophobic residue was chosen to be replaced by tryptophan to minimize changes in polarity and steric effects. To monitor the N-terminal region of the signal sequence, the leucine at position 5 of the wild-type sequence was replaced with tryptophan (5W). The 5 position is adjacent to the dibasic, arginine-lysine sequence at positions 6 and 7 which is one of the features highly conserved among most signal sequences, particularly in prokaryotes (von Heijne, 1984). The valine at position 18, near the C-terminal end of the hydrophobic core, was replaced with tryptophan to serve as a monitor of the central segment of the signal sequence (18W). The 24W peptide replaces the methionine residue at position 24 with tryptophan and was used as a probe of both the extreme C-terminus and the signal peptidase cleavage site.

Effect of Tryptophan Substitutions on Signal Sequence Properties. The effect of the tryptophan substitutions in the wild-type signal sequence has been assayed in vivo (D. Jackson and T. J. Silhavy, personal communication). The 5W and 18W mutations have been found to have a wild-type phenotype for growth and LamB export. The substitution of tryptophan at position 24, however, appears to be lethal. Tryptophan is rarely found within the consensus signal peptidase cleavage site (von Heijne, 1983; Watson, 1984), and the lethality of this mutant may be due to its effect on signal peptidase.

Circular dichroism studies were undertaken to establish the effects of the tryptophan substitutions on the structural properties of the 5W, 18W, and 24W peptides, relative to the wild-type signal peptide (McKnight et al., 1989). In aqueous buffer, the 5W, 18W, and 24W peptides, like the wild-type

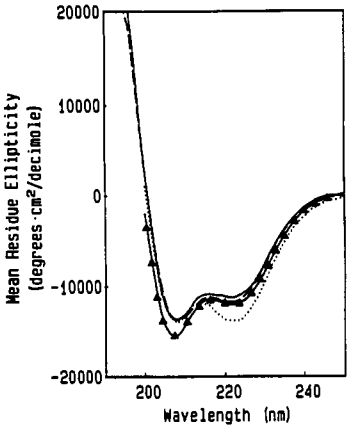

FIGURE 1: Comparison of the CD spectra of tryptophan-containing and wild-type LamB signal peptides in SUVs. Peptide concentrations were 5 μM. SUV lipid concentrations were 1 mM 65% POPE/35% POPG. Solid line, 18W; dashed line, 24W; dotted line, 5W; solid line with triangles, WT.

Table II: Fluorescence Emission Wavelengths (nm) of Tryptophan-Containing Signal Peptides

	λ_{max}		
peptide	5 mM Tris	1 mM LUVs	1 mM SUVs
5W	357	323.5	324
18W	356	326	332.5
24W	358	353	350

peptide, are in a predominantly random conformation (data not shown). In the presence of small unilamellar vesicles of 65% POPG/35% POPE, the CD spectra indicate that the tryptophan-containing peptides adopt approximately the same amount of α-helical structure as the wild-type peptide (Figure 1). However, there is a slight difference in the shape of the CD spectrum of the 5W peptide in SUVs. The minima are of approximately equal intensity in the spectrum of the 5W peptide, whereas the minimum at 208 nm is lower than the minimum at 222 nm for the WT, 18W, and 24W peptides. The small difference in curve shape suggests that there is a slight difference between the conformation of the 5W peptide and the others in SUVs. Despite the small difference in CD spectra of 5W, there are only minor changes in the conformational properties of the LamB signal sequence by the substitution of tryptophan into the 5, 18, or 24 positions. The peptides retain the propensity to adopt helical structure in amphipathic environments.

Fluorescence Emission Blue Shifts Indicate Insertion of Signal Peptides into Lipid Bilayers. Having established that the tryptophan-containing peptides do not have altered conformational properties, steady-state fluorescence spectroscopy was used as a tool to map out the location of these signal peptides in the lipid bilayer. The emission maximum of tryptophan is shifted to lower wavelength (blue shift) as the hydrophobicity of the tryptophan environment increases (Lakowicz, 1983). The insertion of tryptophan into the apolar region of a lipid bilayer leads to significant blue shifts (Le Doan et al., 1986; Jain et al., 1984).

In aqueous buffer, each of these peptides has a fluorescence emission maximum of approximately 357 nm (Table II), similar to the emission of free tryptophan. Upon addition of LUVs which mimic the lipid composition of the *E. coli* membrane (65% POPE/35% POPG), the peptides insert into the bilayer as indicated by the shift of the fluorescence emission maximum to lower wavelength (Table II). The extent of the maximal blue shift varies with the tryptophan position of each peptide. The maximal shift for 24W, 5 nm, is the lowest of

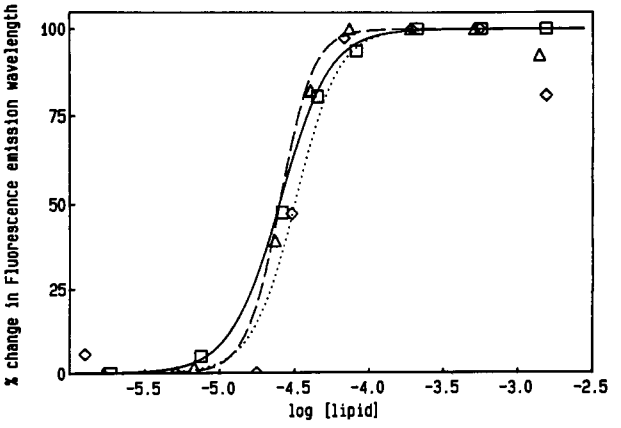

FIGURE 2: Fluorescence emission titration of tryptophan-containing signal peptides with LUVs. The excitation wavelength was 280 nm. The change in tryptophan emission wavelength has been normalized to 100%. Peptide concentrations were 10 μM. LUVs of 65% POPE/35% POPG were added from stock solutions which were also 10 mM in peptide. Open squares and solid line, 5W; open triangles and dashed line, 18W; open diamonds and dotted line, 24W.

the three peptides, implying that the C-terminus of the signal sequence is nearest the bilayer surface. Surprisingly, the emission shift of the tryptophan in the 5W peptide, 33.5 nm, is the largest of the three signal peptides. The 5 position is directly adjacent to the dibasic arginine-lysine sequence at positions 6–7 of the signal sequence. Predictive models place the dibasic region at the bilayer interface, interacting with the negatively charged PG headgroups (von Heijne & Blomberg, 1979; Wickner, 1980; Engelman & Steitz, 1981; Briggs et al., 1986). However, this substantial shift of the tryptophan emission in position 5 suggests that this residue is buried in a highly hydrophobic environment. A possible explanation is that the tryptophan residue is in a hydrophobic pocket formed by the long hydrophobic side chains of arginine and lysine, and thereby shielded from the basic charges and from water. Fluorescence of the 18W peptide also shows a substantial emission shift, 30 nm, indicating the tryptophan in this peptide may be buried in the hydrophobic region of the bilayer. These large changes in fluorescence upon addition of lipid vesicles indicate that the signal peptide not only binds to vesicles but also inserts into the hydrophobic region of the bilayer.

The shift in fluorescence emission maxima was used to monitor the binding of the tryptophan-containing peptides to phospholipid vesicles (Surewicz & Epand, 1984). Figure 2 shows the results of experiments where peptides were titrated with LUVs. With increasing amounts of lipid, there is a corresponding decrease in the fluorescence emission maxima. The peptide concentrations for half-maximal blue shift for the three tryptophan-containing peptides are within experimental error of one another, indicating that the position of the tryptophan residues does not significantly alter the peptide–lipid interaction.

Fluorescence Quenching To Localize Tryptophan Residues in the Bilayer. Fluorescence quenching experiments were undertaken to map the accessibility of the tryptophan residues to soluble and membrane-resident quenchers. Iodide (I^-) was chosen as a soluble quencher to examine the accessibility of the tryptophan residues to the aqueous solvent. Iodide acts as a collisional quencher, quenching the tryptophan fluorescence only when the iodide interacts directly with the tryptophan residue (Lehrer, 1971). A quantitative formalism for this type of quenching is the Stern–Volmer plot. The Stern–Volmer relation is

$$I_0/I = K_{sv}[Q] + 1$$

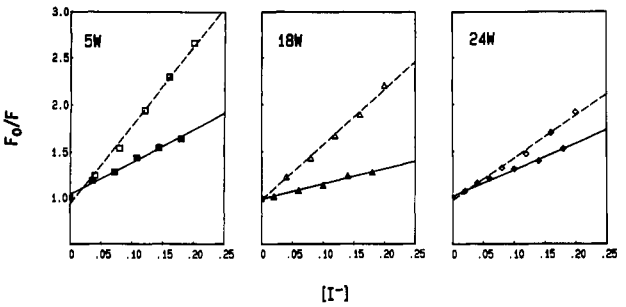

FIGURE 3: Stern–Volmer plots for iodide quenching of tryptophan-containing signal peptides in buffer and 1 mM SUVs. The peptide concentration was 5 μM. The ionic strength was kept constant at 0.2 M salt with NaCl. All solutions were 0.1 mM $Na_2S_2O_3$. F_0/F values are the intensities of the tryptophan emission maxima in the absence of quencher divided by the intensities in the presence of the quencher at the concentration indicated on the x axis. Open symbols, 10 mM Tris, pH 7.3; closed symbols, 1 mM 65% POPE/35% POPG LUVs in 10 mM Tris, pH 7.3.

Table III: Apparent K_{sv} Values of Tryptophan-Containing Peptides Quenched by Iodide[a]

peptide	K_{sv} in Tris	K_{sv} in LUVs
5W	8.5	3.5
18W	5.9	1.6
24W	4.5	2.9

[a] Peptide concentrations were 5 μM. Lipid compositions were 65% POPE/35% POPG at a total lipid concentration of 1 mM. Due to the possibility of some contribution to the slopes of the Stern–Volmer plots from iodide absorbance, we refer to the derived K_{sv} as apparent.

where I_0 is the fluorescence intensity in the absence of any quencher, I is the observed fluorescence at a given quencher concentration [Q], and K_{sv} is the Stern–Volmer quenching constant. If the fluorophore is shielded from the quencher, e.g., by sequestration within a lipid bilayer, then the lower probability of collision will be manifested as a lower K_{sv}. K_{sv} is readily obtained from the slopes of plots of I_0/I vs [quencher]. If a fluorophore is in multiple environments which have differing quencher accessibilities, then deviations from linearity are observed in the Stern–Volmer plots, and a modified form of the Stern–Volmer plot can be used (Lehrer, 1971).

The Stern–Volmer plots for the tryptophan-containing peptides quenched by iodide (Figure 3) are linear, indicating that the tryptophan residue in each peptide is predominantly in a single environment. The difference in slopes in the presence and abscence of vesicles indicates that the peptides are shielded from the quencher by the presence of phospholipid vesicles. Due to the fact that we used an excitation wavelength of 280 nm, there is a small but significant contribution to the observed loss of intensity with increasing iodide concentration from the absorbance of iodide itself. Hence, the K_{sv}'s measured should be considered apparent Stern–Volmer constants. Since the iodide contribution will be linear with [I⁻], the comparison between slopes with and without vesicles is valid. Interestingly, the K_{sv} value (Table III) for the 5W peptide in buffer is significantly greater than that of 24W or 18W. The simplest explanation for this difference in K_{sv} is that the two basic residues next to the tryptophan at position 5 electrostatically attract the negatively charged iodide ion (Lehrer, 1971). Analogously, the negative charge of the C-terminus may shielded the tryptophan of the 24W peptide from the iodide in buffer, resulting in the lower K_{sv} value. The decrease in K_{sv} when the peptides are bound to PE/PG vesicles indicates that the tryptophan residues are sequestered from the iodide.

Table IV: I_0/I Values for Tryptophan-Containing Peptides in 10% Nitroxide Spin-Labeled LUVs[a]

peptide	I_0/I for 5-doxyl-PC	I_0/I for 12-doxyl-PC	I_0/I for 16-doxyl-PC
5W	1.48	1.46	1.76
18W	1.40	1.83	2.12
24W	1.24	1.37	1.54

[a] I_0/I values were calculated from the fluorescence intensity at the emission maximum of each peptide in unlabeled vesicles (10% PSPC) divided by the intensity in the 10% spin-labeled vesicles. Peptide concentrations were 10 μM, and the lipid concentrations were 1 mM.

The 18W peptide exhibits the lowest K_{sv} value in LUVs, indicating that the tryptophan at position 18 is the least accessible to the aqueous-phase quencher. The 5W and 24W peptides have higher K_{sv} values than 18W. However, the change in K_{sv} values for 5W is much greater than that for 24W, suggesting that the tryptophan in the 5W peptide is more shielded from the solvent by interaction with the vesicles than is that of 24W. The interfacial location of the tryptophan of the 5W peptide implied by the iodide quenching suggests that the large blue shift of the tryptophan fluorescence (Table II) is not due to deep insertion but to local effects closer to the interface. The observation of a significant blue shift (10 nm) for this Trp in a bulk solvent [trifluoroethanol (data not shown)] suggests that it may be influenced by neighboring residues, e.g., via a hydrophobic pocket formed by the arginine and lysine side chains.

To examine the extent of insertion of the tryptophan-containing peptides into vesicles, experiments utilizing quenchers covalently attached to the acyl chain of a phospholipid were employed (London, 1982). The bilayer resident quenchers used were nitroxide spin-labeled, acyl chain 1-palmitoyl-(5-, 12-, or 16-doxylstearoyl)phosphatidylcholines. The nitroxide spin-label contains an unpaired electron and acts as a contact quencher of tryptophan fluorescence. The nitroxide label is rather bulky, but it is attractive as a probe because it acts as an efficient quencher, and phospholipids labeled at several different positions along the chain are commercially available. The concentration of the label was kept at 10% in all experiments, to avoid large perturbations of the bilayer.

Fluorescence spectra of the tryptophan-containing peptides show a marked decrease in fluorescence intensity in the presence of nitroxide-labeled LUVs when compared to unlabeled LUVs. For example, Figure 4 shows the spectra of the 5W, 18W, and 24W peptides in LUVs containing no label or containing 16-position spin-labels. The 16-position is near the end of the 18-carbon stearoyl chain and thus is near the center of the bilayer. The I_0/I values for the three tryptophan-containing peptides (Table IV) indicate that the 18W peptide is the most quenched by the 16-position acyl chain quencher followed by 5W; least quenched is the 24W peptide.

Similar results are also seen in LUVs spin-labeled at other acyl chain positions further from the center of the bilayer (Table IV). It is important to note, however, that the exact amount of unpaired spin at a given acyl-chain position may vary significantly from position to position as well as from batch to batch. Chattopadhyay and London used electron spin resonance to determine the actual concentration of unpaired electrons in phospholipids spin-labeled in various positions and found differences of over 10% (Chattopadhyay & London, 1987). This makes comparison of the I_0/I quenching values at differing spin-label positions, even for the same peptide, unreliable. Despite this limitation, examination of the I_0/I values for the quenching of 5W, 18W, and 24W in 10%, 12-doxyl-PC LUVs reveals a similar trend: the 18W position

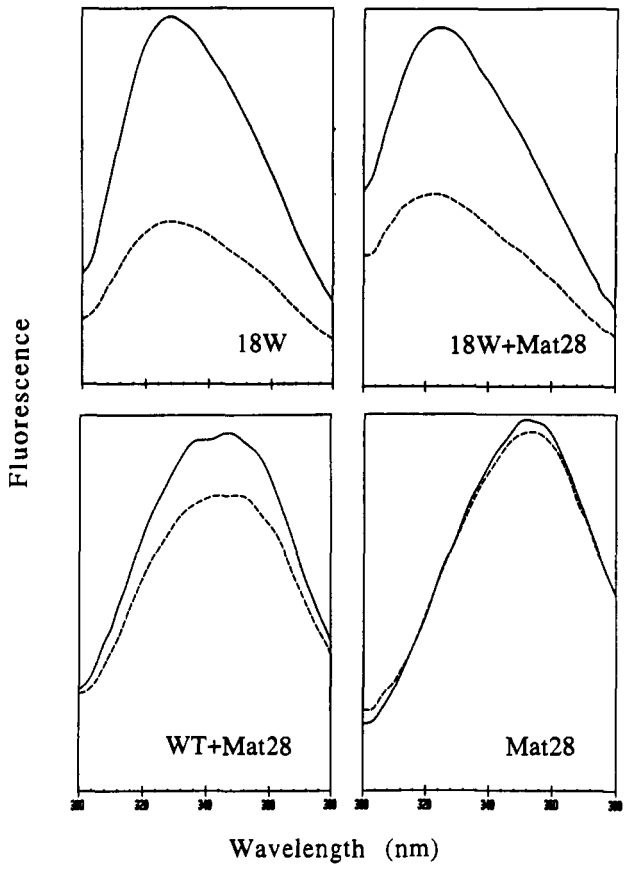

FIGURE 4: Fluorescence of tryptophan-containing signal peptides in 10% 16-position nitroxide-labeled and control LUVs (10% PSPC). Spectra were recorded at 25 °C with an excitation wavelength of 280 nm. Peptide concentrations were 10 μM, and lipid concentrations were 1 mM. Spectra are corrected for scatter by LUVs and smoothed. Note: The asymmetric appearance of these spectra is an instrumental artifact and not indicative of multiple populations, since it occurs also for free tryptophan. Solid lines, control LUVs (55% POPE/35% POPG/10% PSPC). Dashed lines, spin-labeled LUVs [55% POPE/35% POPG/10% 1-palmitoyl-2-(16-doxylstearoyl)-PC].

is quenched most effectively followed by 5W, and least quenched is 24W (Table IV). In 10%, 5-doxyl-PC LUVs, it appears that the tryptophan at position 5 (5W) may be slightly more effectively quenched than the tryptophan in the 18W peptide. This is consistent with a localization of the tryptophan of 5W closer to the interface than that of 18W. Again, the tryptophan at the 24 position (24W) is the least quenched of the three in 10%, 5-doxyl-PC LUVs. These results from membrane-resident quenchers are consistent with those of the iodide quenching experiments and indicate a deep penetration of the signal sequence into the lipid phase.

Discussion

We have used tryptophan as an intrinsic fluorescent probe to establish the nature of the interaction of the LamB signal sequence with phospholipid bilayers. Circular dichroism studies show that the incorporation of tryptophan into the signal peptides does not significantly alter their conformational properties. The placement of the tryptophan at the three different positions also does not change the mode of interaction of the peptides with phospholipid vesicles as seen in similar vesicle titrations. The large blue shifts in the fluorescence emission wavelength of the 5W and 18W peptides, in the presence of lipid vesicles, indicates that they are in a hydrophobic environment within the bilayer, while the 24W peptide is in a more interfacial environment. This topology is reinforced by the relative extent of iodide quenching from the aqueous phase. It appears from these data that the 18-position Trp is least accessible to the aqueous-phase quencher, while the 5- and 24-position Trp's are in a more interfacial environment. The mixing of the external solvent with the solvent entrapped inside the vesicle in the presence of signal peptides in these equilibrium measurements has thus far confounded our efforts to determine the orientation of the inserted peptides with respect to the side of the bilayer from which they were added. We are now applying rapid kinetic methods to determine the inside/outside topology of each tryptophan residue. Further support for insertion of the LamB signal sequence into lipids comes from acyl chain nitroxide spin-label quenching results within the lipid bilayer which indicate that the central region is the most deeply buried in the bilayer.

Each of the methods used to determine the nature of the signal peptide/lipid interaction has its strengths, but no single one yields a clear picture. Blue shifts would seem to suggest that the Trp at position 5 is most buried; however, the shift in emission maximum is very sensitive to solvation of the fluorophore. Given the consistent picture from both iodide and nitroxide quenching that the 18-position Trp is more sequestered within the bilayer than the 5-position Trp (and both are clearly more buried than the 24-position Trp), we conclude that the local environment is contributing to the large blue shift of the Trp at position 5, and we caution those using blue shifts as probes of membrane interactions to consider possible local environmental influences.

The topology data are consistent with the signal peptide spanning the bilayer in a transmembrane fashion or inserted into the vesicle in a looped structure. From the results of quenching by both iodide and nitroxide, we find unlikely the possibility that the peptide is oriented parallel to the membrane surface. Further topology experiments are required to differentiate rigorously the looped and transmembrane possibilities. however, NMR studies of the LamB signal sequence in a helix-promoting environment have shown the most stable, helical region of the peptide is within its hydrophobic core (Bruch et al., 1989). The stable helical region extends from leucine-10 to glutamine-20. The high helical content of the signal sequence in phospholipid vesicles (70%), presumably within the hydrophobic core, argues against a hairpin conformation which would require a turn within this region.

The spontaneous insertion of the signal sequence into the lipid phase of membranes concomitant with the adoption of helical structure may be an important event in protein secretion. In current models for bacterial protein export (Bieker & Silhavy, 1990; Hartl et al., 1990), the nascent chain is recognized first by SecB. SecB is a small soluble protein that functions as a chaperone to maintain the precursor in an export-competent conformation (Liu et al., 1989; Kumamoto, 1990). The signal sequence is then recognized by SecA, a large soluble ATPase (Oliver, 1990). This interaction probably occurs in the cytoplasm, which clearly is an *aqueous* location. SecA then pilots the precursor to the membrane, most likely via interaction with the integral membrane protein SecE. At this stage, we believe that the signal sequence inserts into the membrane bilayer. Suppressor mutations that alter signal sequence recognition by both SecE and SecY change amino acid residues located within transmembrane helices of both proteins (Ito, 1990; Bieker & Silhavy, 1990). This implies that both SecE and SecY interact with the signal sequence after membrane insertion. We believe that the transition from aqueous to membrane phases is driven by the strong tendency of signal sequences to insert spontaneously into the lipid bilayer. Furthermore, the spontaneous insertion of the signal

sequence into the lipid phase restricts the nascent chain to two-dimensional diffusion and most likely orients it appropriately for the next protein recognition steps, allowing formation of a functional translocator and export.

ACKNOWLEDGMENTS

We thank Tom Silhavy and Dolores Jackson for helpful discussion and for unpublished results, and Jeff Jones for critical reading of the manuscript. We also thank Anita Sgrignoli and Sarah Stradley for peptide synthesis and Khuan Ng and Candace Millhouse for amino acid analysis and peptide sequencing.

Registry No. POPE, 26662-94-2; POPG, 81490-05-3; PSPC, 59403-51-9.

REFERENCES

Ames, B. N. (1966) *Methods Enzymol. 8*, 115–118.

Barany, G., & Merrifield, R. B. (1979) in *The Peptides* (Gross, E., & Meienhofer, J., Eds.) Vol. 2, pp 1–284, Academic Press Inc., New York.

Batenburg, A. M., Brasseur, R., Ruysschaert, J.-M., van Scharrenburg, G. J. M., Slotboom, A. J., Demel, R. A., & de Kruijff, B. (1988a) *J. Biol. Chem. 263*, 4202–4207.

Batenburg, A. M., Demel, R. A., Verkleij, A. J., & de Kruijff, B. (1988b) *Biochemistry 27*, 5678–5685.

Bieker, K. L., & Silhavy, T. J. (1990) *Cell 61*, 833–842.

Briggs, M. S., Cornell, D. G., Dluhy, R. A., & Gierasch, L. M. (1986) *Science 233*, 206–208.

Bruch, M. D., McKnight, C. J., & Gierasch, L. M. (1989) *Biochemistry 28*, 8554–8561.

Chattopadhyay, A., & London, E. (1987) *Biochemistry 26*, 39–45.

Chen, L., & Tai, P. C. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 4384–4388.

Collier, D. N., Bankaitis, V. A., Weiss, J. B., & Bassford, P. J., Jr. (1988) *Cell 53*, 273–283.

Connolly, T., & Gilmore, R. (1989) *Cell 57*, 599–610.

Cornell, D. G., Dluhy, R. A., Briggs, M. S., McKnight, C. J., & Gierasch, L. M. (1989) *Biochemistry 28*, 2789–2797.

Emr, S. D., Hanley-Way, S., & Silhavy, T. J. (1981) *Cell 23*, 79–88.

Engelman, D. M., & Steitz, T. A. (1981) *Cell 23*, 411–422.

Erickson, B. W., & Merrifield, R. B. (1976) in *The Proteins* (Neurath, H., & Hill, R. L., Eds.) Vol. 2, pp 255–527, Academic Press Inc., New York.

Fiske, C. H., & SubbaRow, Y. (1925) *J. Biol. Chem. 66*, 375–400.

Geller, B. L., & Wickner, W. (1985) *J. Biol. Chem. 260*, 13281–13285.

Gierasch, L. M. (1989) *Biochemistry 28*, 923–930.

Hartl, F.-U., Lecker, S., Schiebel, E., Hendrick, J. P., & Wickner, W. (1990) *Cell 63*, 269–279.

Hartmann, E., Wiedmann, M., & Rapoport, T. A. (1989) *EMBO J. 8*, 2225–2229.

Ito, K. (1984) *Mol. Gen. Genet. 197*, 204–208.

Ito, K. (1990) *J. Bioenerg. Biomembr. 22*, 353–368.

Jain, M. K., Rogers, J., Simpson, L., & Gierasch, L. M. (1984) *Biochim. Biophys. Acta 816*, 153–162.

Killian, J. A., de Jongh, A. M. Ph., Bijvelt, J., Verkleij, & de Kruijff, B. (1990a) *EMBO J. 9*, 815–819.

Killian, J. A., Keller, R. C. A., Struyve, M., de Kroon, A. I. P. M., Tommassen, J., & de Kruijff, B. (1990b) *Biochemistry 29*, 8131–8137.

Kumamoto, C. (1990) *J. Bioenerg. Biomembr. 22*, 337–352.

Lakowicz, J. R. (1983) *Principles of Fluorescence Spectroscopy*, Plenum Press, New York.

Le Doan, T., El Hajji, M., Rebuffat, S., Rajesvari, M. R., & Bodo, B. (1986) *Biochim. Biophys. Acta 858*, 1–5.

Lehrer, S. S. (1971) *Biochemistry 10*, 3254–3263.

Liu, G., Topping, T. B., & Randall, L. L. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 9213–9217.

London, E. (1982) *Mol. Cell. Biochem. 45*, 181–188.

Mayer, L. D., Nelsestuen, G. L., & Brockman, H. L. (1983) *Biochemistry 22*, 316–321.

McKnight, C. J., Briggs, M. S., & Gierasch, L. M. (1989) *J. Biol. Chem. 264*, 17293–17297.

Oliver, D. B., Cabelli, R. J., & Jarosik, G. P. (1990) *J. Bioenerg. Biomembr. 22*, 311–336.

Schmidt, M. G., Rollo, E. E., Grodberg, J., & Oliver, D. B. (1988) *J. Bacteriol. 170*, 4304–4314.

Shinnar, A. E., & Kaiser, E. T. (1984) *J. Am. Chem. Soc. 106*, 5006–5007.

Surewicz, W. K., & Epand, R. M. (1984) *Biochemistry 23*, 6072–6077.

Verner, K., & Schatz, G. (1988) *Science 241*, 1307–1313.

von Heijne, G. (1983) *Eur. J. Biochem. 133*, 17–22.

von Heijne, G. (1984) *EMBO J. 3*, 2315–2318.

von Heijne, G., & Blomberg, C. (1979) *Eur. J. Biochem. 97*, 175–181.

Walter, P., & Blobel, G. (1983) *Cell 34*, 525–533.

Watanabe, M., & Blobel, G. (1989) *Cell 58*, 695–705.

Watson, M. E. E. (1984) *Nucleic Acids Res. 12*, 5145–5164.

Wickner, W. (1980) *Science 210*, 861–868.

Wickner, W. (1988) *Biochemistry 27*, 1081–1086.