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# MINI-REVIEW

# Biophysical Studies of Signal Peptides: Implications for Signal Sequence Functions and the Involvement of Lipid in Protein Export

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## Abstract

This review discusses efforts to understand the mode of action of signal sequences by biophysical study of synthetic peptides corresponding to these protein localization signals. On the basis of reports from several laboratories, it is now clear that signal peptides may adopt a variety of conformations, depending on their local environment. In membrane-mimetic systems like detergent micelles or lipid vesicles, they have a high tendency to form α helices. Ability to take up a helical conformation appears to be required at some point in the function of a signal sequence, since some peptides corresponding to export-defective signal sequences display reduced helical potential. By contrast, functional signal sequences share a high capacity to adopt α helices. High affinity for organized lipid assemblies, like monolayers or vesicles, is also a property of functional signal sequences. This correlation suggests a role for direct interaction of signal sequences with the lipids of the cytoplasmic membrane *in vivo*. Supporting this role are studies of the influence of signal peptides on lipid structure, which reveal an ability of these peptides to pertub lipid packing and to alter the phase state of the lipids. Insertion of the signal sequence *in vivo* could substantially reduce the barrier for translocation of the mature chain. Lastly, synthetic signal peptides have been added to native membranes and found to inhibit translocation of precursor proteins. This approach bridges the biophysical and the biochemical aspects of protein export and promises to shed light on the functional correlates of the properties and interactions observed in model systems.

**Key Words:** Signal sequence; conformation; lipid; protein export.

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## Introduction

While the importance of the signal sequence in protein secretion is unequivocably established, a detailed understanding of its roles and interactions remains elusive. It has been clearly demonstrated in eukaryotes that a nascent chain with its transient signal sequence is recognized by a proteinaceous complex, the signal recognition particle (SRP), resident in the cytoplasm (Kurzchalia *et al.*, 1986; Krieg *et al.*, 1986). The identity of the corresponding species in prokaryotes is as yet controversial (see, for example, Watanabe and Blobel, 1989; Lill *et al.*, 1989), but it is agreed that a similar mechanism is operative. According to the paradigm of the signal hypothesis, the nascent chain is next targeted to the appropriate membrane (endoplasmic reticulum in eukaryotes or plasma membrane in prokaryotes) via a specific binding of the recognition particle and its "receptor" (Meyer *et al.*, 1982; Gilmore *et al.*, 1982). Whether there is at this point a binding of the nascent chain bearing its signal (or leader) sequence to another protein receptor and whether translocation occurs through a proteinaceous pore have not yet been established. Many researchers support a model in which both binding and translocation are protein-mediated, and the secY(prlA) gene product is a favored candidate for these functions in prokaryotes.

On the other hand, it has been suggested from the earliest studies that signal sequences may facilitate membrane insertion and translocation of the secretory protein via direct interactions with the phospholipids of the bilayer. Von Heijne and Blomberg's direct transfer model (1979), Engelman and Steitz's helical hairpin hypothesis (1981), Bedouelle and Hofnung's hydrophobic axis length proposal (1981), Inouye and Halegoua's loop model (1980), and Wickner's membrane trigger hypothesis (1980) all postulate that the nascent chain interacts directly with the bilayer. A major motivation for all of these proposals was the highly hydrophobic nature of the signal sequence. Ironically, until recently there had been no experimental studies of signal peptides that could test these expectations and refine the description of signal sequence properties to shed light on their potential roles in protein export. In the last few years, this approach has been productively invoked in a variety of cases.

This review summarizes the results of analyses of peptides corresponding to signal sequences of exported proteins and critically considers their implications. Not surprisingly, one of the general findings is that indeed signal peptides show a strong tendency to interact with phospholipids. The significance of this behavior is discussed, and related results concerning the involvement of lipids in protein export are reviewed.

## Conformations of Signal Peptides

Signal sequences have strong similarities in residue types, despite their lack of primary sequence homology, suggesting that their functions may be dependent on the adoption of specific secondary structural motifs. A defined secondary structure may be essential for proper recognition of signal sequences by cytosolic binding factors. Furthermore, the subsequent interaction with the secretory membrane, via binding to lipid and/or integral membrane proteins, may be quite specific with regard to signal sequence structure. Thus, it is of great interest to understand the conformational preferences of these sequences in a variety of environments. The studies described have been carried out on signal sequences as isolated peptides or, in some cases, on peptides resembling signal sequences.

Secondary structure prediction algorithms attribute a high penchant for formation of either $\beta$ structure or $\alpha$ helix to the central core of signal sequences. On the whole, these predictions have been supported by circular dichroism (CD), infrared (IR), and most recently, nuclear magnetic resonance (NMR) spectroscopy studies of a number of synthetic signal peptides. As the results described below illustrate, one can expect signal sequences to be conformationally responsive to their milieu and to be able to adopt any of a range of conformations. Shinnar and Kaiser (1984) examined the conformation of the 23-residue signal sequence of phage M13 coat protein by CD. In polar solvents, the peptide adopted little secondary structure. A very similar result had been obtained for a 19-residue peptide modeled on the signal sequence of pretrypsinogen (Austen and Ridd, 1981). However, in solutions of the polyfluorinated alcohols, trifluoroethanol (TFE) or hexafluoroisopropanol (HFIP), a significant amount of $\alpha$ helical content was observed for these peptides, as well as for peptides resembling the signal sequences of lysozyme and lipoprotein (Reddy and Nagaraj, 1985). These solvents promote the formation of intramolecular hydrogen bonds, and thereby favor $\alpha$ helix. A 29-residue peptide consisting of the 23-residue signal sequence plus the polar 6-residue segment of parathyroid hormone (the "prepro" region) adopted primarily a $\beta$ conformation in aqueous solution, but was likewise observed to become partially helical in TFE and HFIP (Rosenblatt *et al.*, 1980). In contrast to these results, Katakai and Iizuka (1984) found that peptides containing 13- or 14-residue fragments of three eukaryotic signal peptides (preimmunoglobulin light chain, pretrypsinogen, and pre-$\beta$-lactoglobulin) have random conformations in HFIP, become $\alpha$-helical in mixtures of HFIP and nonfluorinated alcohols, and adopt $\beta$ structure in aqueous HFIP.

Batenburg *et al.* (1988a) examined the conformational behavior of different regions of a signal sequence using peptides corresponding to

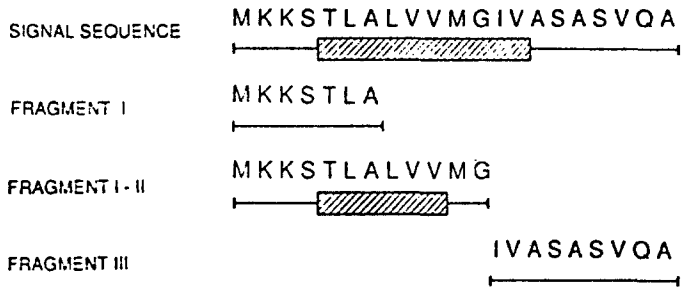


**Fig. 1.** Primary and predicted secondary structure of the PhoE signal sequence and its studied fragments. Secondary structure was predicted according to Chou and Fasman (1974); ——, no structure predicted; ▨, $\beta$ strand. From Batenburg *et al.*, (1988a).

fragments and to the full-length PhoE signal sequence (Fig. 1). In aqueous solution, CD results indicated that the wild type (WT) peptide aggregates in a 100% $\beta$-structure, while fragment I–II adopted a random structure (Table I). However, in TFE or 0.8% SDS, a significant amount of $\alpha$ helix was observed for both peptides. Without fragment II, the N-terminal fragment I peptide was a random coil in all three solvents, and the C-terminal fragment III was unstructured in 0.8% SDS and gave an unusual spectrum in TFE. These results argue that the hydrophobic core region, represented here by fragment II, is essential for adoption of $\alpha$ helix in signal peptides.

Taken together, the above studies demonstrate that signal peptide conformation varies widely depending on environment. It seems likely that the tendency to adopt alternate conformations in different cellular environments confers on these sequences the ability to serve multiple roles in protein secretion. For instance, a cytoplasmic factor (SRP or analog) may

**Table I.** CD-Based Secondary Structure Analysis of PhoE Signal Sequence and Fragment I–II

| Peptide | Solvent | Analysis based on spectral shape % | | | Analysis based on spectral intensity % | | |
|---|---|---|---|---|---|---|---|
| | | $\alpha$ | $\beta$ | Random | $\alpha$ | $\beta$ | Random |
| Signal sequence | Water | 0 | 100 | 0 | 0 | 100 | 0 |
| | TFE[a] | 60 | 0 | 40 | 62 | 2 | 36 |
| | 0.8% SDS | 80 | 8 | 12 | 75 | 9 | 16 |
| Fragment I–II | Water | 0 | 0 | 100 | 0 | 0 | 100 |
| | TFE | 50 | 0 | 50 | 43 | 4 | 53 |
| | 0.8% SDS | 63 | 0 | 37 | 41 | 2 | 57 |

[a]TFE, trifluoroethanol.

preferentially interact with an unstructured sequence in the cytoplasm. Also, the ability to adopt a given secondary structure may be critical for proper interaction with the plasma membrane. Despite some variations in conformational behavior from one example to another, the ability to fold into a partially α-helical conformation in a nonpolar environment seems to be a general property of signal peptides described above. However, these results do not reveal how conformational preferences are related to signal sequence functions.

In order to define structure/function relationships among signal sequences more systematically, our laboratory has examined the conformational and lipid binding properties of a family of peptides derived from the signal sequences of wild type and export-defective mutants of the gene product of the *E. coli* outer membrane protein LamB (λ-receptor or maltoporin). Briggs and Gierasch (1984) examined the conformation of peptide fragments corresponding to the WT and the first three mutant LamB signal sequences which are illustrated in Fig. 2, and which were originally isolated by Emr and Silhavy (1983). The WT sequence contains an uninterrupted stretch of seven hydrophobic amino acids flanked by a proline and a glycine. Chou–Fasman algorithms predict a high helical probability for this segment (Chou and Fasman, 1974). An export-defective deletion mutant Δ78 is missing four residues from the hydrophobic core region, which brings the helix-breaking residues proline and glycine to within three residues of each other, and this sequence is not expected to fold into a helix. In the two export-competent pseudorevertants, point mutations occurred which replaced the helix-breaking Pro or Gly residues by helix-promoting residues. In Δ78r1, the glycine residue is mutated to a cysteine, resulting in restoration of 50% secretion activity. In Δ78r2, proline is replaced by a leucine, resulting in 90% secretion activity. These changes restore predicted helicity to these peptides. Emr and Silhavy (1983) thus suggested from this analysis of the sequences that the ability of the point mutation pseudorevertants to restore function was due to the

| | |
|---|---|
| WT | MMITLRKLPLAVAVAAGVMSAQAMA |
| Δ78 | MMITLRKLP----VAAGVMSAQAMA |
| Δ78r1 | MMITLRKLP----VAACVMSAQAMA |
| Δ78r2 | MMITLRKLL----VAAGVMSAQAMA |
| G17R | MMITLRKLPLAVAVAARVMSAQAMA |
| A13D | MMITLRKLPLAVDVAAGVMSAQAMA |

**Fig. 2.** Amino acid sequences of the LamB signal peptides. The underlined segments represent the fragments studied by Briggs and Gierasch (1984).

enhanced capacity of these sequences to adopt helices in the hydrophobic core region.

Results from studies of peptides corresponding to these sequences supported this proposed relationship between the observed secretion phenotype and conformational tendencies. The LamB signal peptide fragments consisted of the hydrophobic core plus several residues on either side of the proline and glycine to allow for helix nucleation in the pseudorevertants. The basic residues (Arg 6 and Lys 7) were present as they may enhance interaction of the peptides with negatively charged headgroups of lipidlike molecules and should enhance solubility. Environments examined included aqueous buffers as a polar environment, 20% TFE as a nonpolar, helix-promoting environment, and micelles comprised of either 40 mM sodium dodecyl sulfate (SDS) or lysolecithin as environments which mimic phospholipid membranes. All of the peptides were observed to take up a predominantly random conformation in aqueous buffer. In 40 mM SDS, the wild type and revertant peptides adopt significant $\alpha$-helical structure. However, the conformation of the deletion mutant shows little change between water and SDS. Similar structural trends were observed in 20% TFE and 25 mM lysolecithin.

These results suggest that the ability of a signal peptide to adopt $\alpha$-helical structure in a nonpolar environment may be crucial to proper function, either for signal sequence interaction with the apolar region of the membrane or for binding to a protein of the export apparatus. However, these studies do not reveal if helical content is sufficient to define a functional signal sequence. In recent work (McKnight *et al.*, 1989), the full-length peptides corresponding to the LamB sequences described above and, in addition, two peptides corresponding to export-impaired sequences with charged residues in the hydrophobic core region (Stader *et al.*, 1986) were examined (Fig. 2). The export-defective mutant peptide, A13D, has an aspartate residue at position 13 in the middle of the hydrophobic segment. Aspartate is not generally considered a helix breaker (Chou and Fasman, 1974), but the charge lowers the overall hydrophobicity of this segment. Replacement of Gly 17 with arginine (G17R) near the end of the hydrophobic segment causes a less severe, kinetic export defect. This peptide directs the export of as much LamB as the wild type signal sequence but at a slower rate. These results suggest that decreased hydrophobicity in the signal sequence core region can inhibit secretion activity, although the predicted tendency to adopt helical conformation is not substantially reduced.

The fraction of $\alpha$ helix observed for these peptides in 40 mM SDS, along with their *in vivo* activity, is given in Table II. The export-competent peptides generally have the highest proportion of $\alpha$ helix level. The semifunctional revertant ($\Delta$78r1) and the nonfunctional deletion mutant have the lowest

**Table II.** Summary of the Activities and Biophysical Parameters of Signal Peptides

| Name | *In vivo* activity[a] (%) | α-Helix content[b] (%) | Monolayer insertion[c] (nM) |
|---|---|---|---|
| WT | 100 | 70 | 10.8/46 |
| Δ78 | 0 | 35 | 2.5/125 |
| Δ78r1 | 50 | 40 | ND |
| Δ78r2 | 90 | 75 | 10.2/177 |
| G17R | 40 | 70 | 10.6/83 |
| A13D | 10 | 60 | 9.6/536 |

[a]Activity is the amount of LamB expressed in the outer membrane in 4 min (Stader *et al.*, 1986).
[b]α-Helix content calculated from CD spectra in 40 mM SDS, fitted to Greenfield and Fasman (1969) reference data .
[c]Membrane insertion: $\Delta\pi_{max}$ (dynes/cm)/$K_D$. Monolayers were composed of 65% POPE and 35% POPG.

helical contents. However, the export-defective A13D peptide has an α-helix comparable to the functional peptides. Thus, while α-helical content seems necessary for export capability, it is clearly not sufficient to ensure proper signal sequence function. The A13D result strongly supports the assertion that hydrophobicity in the core region is also critically related to function (Chou and Kendall, 1990), which suggests a lipid binding role for the signal sequence.

The structure and stability of the LamB WT, Δ78, Δ78r1, and Δ78r2 signal peptides have recently been examined in more detail by two-dimensional nuclear magnetic resonance spectroscopy (2D-NMR) (Bruch *et al.*, 1989; Bruch and Gierasch, 1990). This technique allows determination of specific residues which take up a helix, as opposed to CD which gives an overall conformational behavior. The conformational properties of the peptides were examined in 50% TFE/$H_2O$. The proportion of α helix in this solvent paralleled that found in phospholipid vesicles according to CD (see below). Thus, this mixture was chosen as a reasonable environment to mimic that of a membrane.

The WT peptide was found to take up an α-helical conformation which is in a dynamic equilibrium with a more random structure. Loss of secondary structure was evident upon increasing temperature. Residues 10–18 in the hydrophobic core region existed as a helix up to 50°C. Helical structure propagated to the C-terminal region of the molecule at 25°C, with approximately 17 residues adopting a helical conformation at this temperature. However, the temperature had to be decreased to 5°C before any helix was observed in the N-terminal region. These results imply that the helix-breaking power of the proline residue is greater than that of the glycine.

NMR results showed that the pseudorevertant signal peptides have approximately 12 residues in a helical conformation at 25°C in aqueous

trifluoroethanol (50%). The functional signal peptide derived from pseudorevertant Δ78r2 maintained a stable helix in the hydrophobic core region up to 50°C. The semifunctional signal peptide from the mutant Δ78r1 displayed a few turns of helix at this temperature, but no persistent helical conformation was observed. By contrast, the nonfunctional deletion mutant signal peptide (Δ78) exhibited no secondary structure at 50°C. At 25°C, approximately seven residues were in a helical conformation. These results suggest that helix *stability* in the hydrophobic core region is crucial to proper signal sequence function. However, it cannot be ruled out that function also requires a critical *length* of helix.

## Interaction of Signal Peptides with Lipid

The above studies demonstrate that functional signal peptides have similar conformational preferences. Export-active peptides have a marked tendency to fold into partial α-helical structures in nonpolar environments; helix appears to be most favored in the hydrophobic core. However, the studies on the LamB A13D mutant demonstrate that the ability to adopt helical conformation is not sufficient to ensure export competence. The observation that decreased hydrophobicity in the core region substantially reduces activity suggests that the ability of signal sequences to interact productively with lipid may be essential for function. At face value, this correlation supports those models for protein insertion and translocation that envision direct transfer of secretory proteins through the lipid phase. Several studies of synthetic signal peptides have explored in greater detail their affinities and modes of interaction with phospholipid monolayers and bilayer vesicles.

We have studied the affinity of the LamB signal peptide family for phospholipid monolayers and their mode of interaction by surface tensiometry. In this method, a lipid monolayer is spread onto a buffer surface to a desired lateral pressure (which corresponds to tightness of packing). The interaction of peptides with this deposited monolayer can then be monitored by changes in monolayer surface pressure upon titration of the peptides into the aqueous subphase. Peptides which interact with the headgroups of the phospholipid monolayer cause only a small (1–3 dynes/cm) increase in the monolayer surface pressure (Mayer *et al.*, 1983); peptides which insert into the acyl chain region cause much larger changes in monolayer surface pressure (Bougis *et al.*, 1981). Affinities of peptides for the lipid monolayer (apparent $K_D$'s) are reflected in the concentrations of peptides required for half-maximal surface pressure change. Thus, differential interactions of peptides with lipid monolayers can be examined.

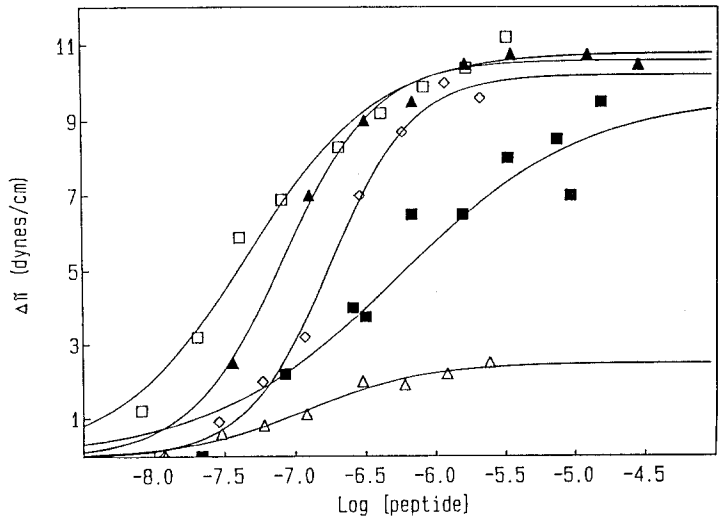


**Fig. 3.** Insertion of LamB signal peptides into monolayers. The initial surface pressure was 23.5 ± 0.5 dynes/cm. Monolayers were composed of 65% POPE and 35% POPG. □, WT; △, Δ78; ◇, Δ78r2; ▲, G17R; ■, A13D. From McKnight *et al.* (1989).

Surface pressure changes upon interaction of LamB signal peptides with a phospholipid monolayer that mimics the composition and surface charge of the *E. coli* plasma membrane are shown in Fig. 3 (McKnight *et al.*, 1989). The wild type and functional pseudorevertant signal peptides cause relatively large increases in surface pressure (ca. 10 dynes/cm). In contradistinction to these results, the nonfunctional deletion mutant causes only a 2.5 dynes/cm surface pressure change, which implies that this peptide does not insert into the acyl chain region of the monolayer. However, the apparent $K_D$ for monolayer binding of this peptide is similar to that observed for the functional peptides (Table II). Thus, the deletion in the core region apparently affects the nature of the interaction of the peptide with the lipid monolayer, rather than altering affinity. The peptide corresponding to the export-defective point mutation A13D causes a somewhat lower maximum pressure change than that observed for the functional peptides. Also, the affinity of this peptide is substantially lower than that observed for the functinal peptides. It can therefore be concluded that peptides corresponding to export-defective sequences either do not insert into the hydrocarbon region of these lipid monolayers, or do so only at much higher concentrations than are necessary for export-active peptides. A similar result was obtained for the interaction of the previously discussed LamB fragment peptides (Fig. 2) with egg PE/PG monolayers (Briggs *et al.*, 1985).

Phospholipid monolayers and bilayers exhibit many differences in their physical properties. It is possible that peptide-monolayer interactions are distinct from the behavior of bilayer systems. Thus, we employed fluorescence spectroscopy to examine signal peptide–lipid interactions with bilayer vesicles. This technique has been used extensively to examine the interactions of proteins and peptides with lipid vesicles. The spectral properties of tryptophan are sensitive to local environment. The emission maximum is observed to shift to lower wavelengths (blue shift) on displacement from aqueous buffer to a relatively nonpolar environment. Thus, the binding of a Trp-containing peptide to a phospholipid vesicle can be monitored by changes in emission maximum (Surewicz and Epand, 1984). Also, quenching of Trp fluorescence by membrane-resident quenchers located at specific sites along the lipid acyl chain can provide information regarding the topology of the peptide within the vesicle bilayer (London, 1982).

We have recently exploited these techniques in order to examine in greater detail the interaction of signal peptides corresponding to the LamB WT and deletion mutant sequences with phospholipid vesicles (McKnight, 1990; McKnight, Rafalski, and Gierasch, unpublished results). LamB signal peptide mutants were synthesized containing Trp at positions 5, 18, or 24. These mutations resulted in replacement of a relatively large hydrophobic residue by Trp in each case to minimize potential structural perturbation. Mutants containing Trp at various loci permit determination of the position of different segments of the peptide with respect to the bilayer surface. Also, the deletion mutant was synthesized with Trp at position 18 of the WT sequence. Analogously to the monolayer studies, the extent of penetration of the Trp-containing region of the peptide is defined by the maximally attained blue shift. Also, peptide affinity for the vesicle is given by the peptide concentration at which the half-maximal change is observed.

The change in emission wavelength upon vesicle binding (Fig. 4) indicates significant alteration of the Trp environment. The Trp-containing peptides exhibit relatively high binding affinities (Table III). Also, the Trp fluorescence of the 5W and 18W peptides shows large blue shifts (ca. 30 nm), indicating that these Trp residues are sampling the hydrophobic bilayer interior. By contrast, the Trp fluorescence of the LamB 24W mutant undergoes only a slight shift in emission maximum. Thus, the C-terminal region of the peptide is most likely associated with the bilayer near the interfacial region. This result is consistent with either a transmembrane arrangement of the peptide, or a looped conformation with both termini on the same side of the vesicle. However, the relatively high proportion of helix in these peptides in vesicles is more consistent with a linear, transmembrane orientation of the peptide.

The fluorescence of the Trp mutant corresponding to the nonfunctional deletion mutant exhibits a relatively small blue shift upon interaction with

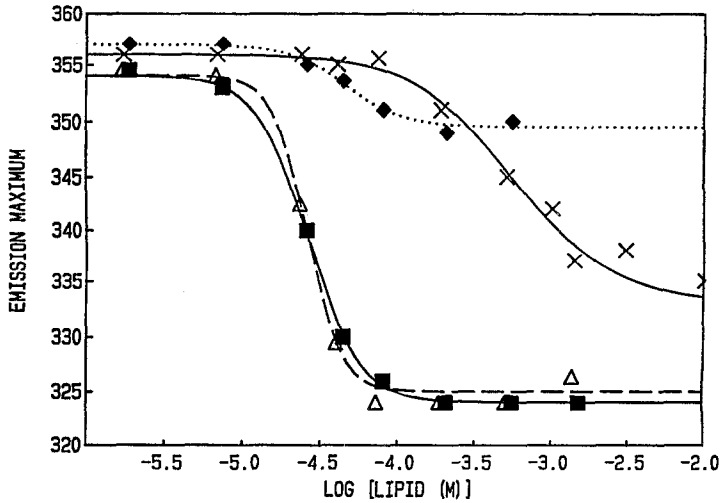


**Fig. 4.** Trp emission maximum as a function of lipid concentration for LamB signal peptides. Vesicles were composed of 65% POPE and 35% POPG. ■, 5W; △, 18W; ◆, 24W; ×, Δ78/18W.

bilayers. This result supports the conclusion obtained from the monolayer studies: that the peptide does not significantly penetrate the bilayer hydrocarbon region. However, the apparent binding affinity is significantly lower than that for the functional peptides. This result differs from that obtained in the monolayer experiments, where similar affinities were observed. Differences in properties of vesicles and monolayers, such as surface curvature and dielectric constant, may influence relative peptide affinity in this series. Nonetheless, both techniques demonstrated that the ability to interact with the hydrocarbon region of lipid is a hallmark of functional signal sequences.

Membrane-resident quenching with covalently attached spin labels at the 5, 12, and 16 positions of the lipid acyl chains provides further evidence that these peptides insert into the hydrocarbon region of a bilayer. Relative quenching efficiences ($I_0/I$) for 5W, 18W, and 24W (Table III) show that each

**Table III.** $I_0/I$ Values for Tryptophan-Containing Peptides

| Peptide | $I_0/I^a$ | | |
|---|---|---|---|
| | 5-Doxyl PC[b] | 12-Doxyl PC | 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] Values of $I_0/I$ were calculated from the fluorescence intensity at the emission maximum of each peptide in unlabeled vesicles divided by the intensity in the spin-labeled vesicles.
[b] Large unilamellar vesicles were composed of 10% nitroxide, 55% POPE and 35% POPG.

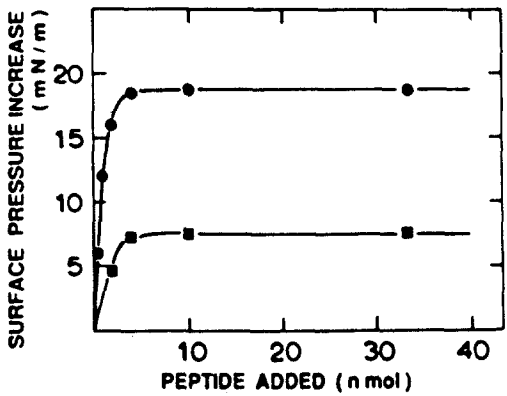


**Fig. 5.** Surface pressure increase of monolayers of PC and cardiolipin as a function of the amount of peptide injected into the subphase. ●, cardiolipin; ■, SoyPC. From Batenburg *et al.* (1988b).

peptide is most efficiently quenched by the 16-position probe. The relative quenching by the 12- and 16-position probes follows the series 18W > 5W > 24W; the 5-position probe quenches 18W and 5W with about the same efficiency, but still greater than 24W. These results are consistent with those obtained from the Trp emission shift experiments, which demonstrated that the Trp at position 18 interacts most effectively with the lipid hydrocarbon region, followed next by that at the 5 position; the Trp at the 24 position again seems most interfacial.

The studies with the LamB peptide family illustrate the dependence of peptide structural properties on interaction of the peptides with phospholipid. Batenburg *et al.* (1988b) examined the interaction of PhoE peptides (Fig. 1) with phospholipid monolayers and bilayers as a function of lipid composition and ionic strength of the medium. The interaction of the WT peptide with cardiolipin monolayers is much stronger than with soy phosphatidylcholine (soyPC) monolayers, as shown in Fig. 5. This result implies that there is a strong electrostatic component to the binding. However, there is likely to be a sizable hydrophobic component to the interaction, and the necessity for a relatively high salt concentration to inhibit binding significantly is consistent with this as well. The effect of lipid phase structure and unsaturation was examined by analyzing the interaction of this peptide with phosphatidylserine (PS) monolayers under a variety of conditions. The interaction of the peptide was similar for liquid crystalline monolayers, regardless of the degree of saturation. However, the peptide was observed to interact less efficiently with gel-phase dimyristoyl PS monolayers at 10°C. Hence, lipid phase state, but not unsaturation as such, affects the nature of peptide binding.

The binding of the peptide fragments shown in Fig. 1 was also examined. Fragment III, which lacks a net charge, shows no specificity and weak interaction with all monolayers studied. Fragment I and fragment I–II showed behavior similar to the wild type sequence in that they interacted much more readily with cardiolipin monolayers than with those composed of soyPC. Fragment I–II, however, which contains the hydrophobic core region, caused a larger surface pressure change. This result further suggests that electrostatic interaction of the peptide N-terminal region with negatively charged phospholipids is a prerequisite to efficient interaction between the peptide and the monolayer. However, the presence of the hydrophobic core region is necessary for signal peptide insertion into the apolar acyl chain region of lipids.

## Conformation of Signal Peptides Bound to Lipid

The above studies indicate that the ability of signal peptides to bind and perturb lipid correlates with their secretion activity. Also, these peptides are very conformationally sensitive to their local environment. It is thus quite likely that signal sequences adopt different conformations depending on the site of membrane binding. For instance, surface binding, via electrostatic interactions with lipid headgroups, may induce a conformation much different from that assumed upon peptide insertion. The ability of signal sequences to alter their conformation at various stages of membrane interaction may be crucial to their function. The lipid-induced signal sequence conformation may facilitate binding of the mature protein to the lipid and subsequent translocation directly through the lipid phase. Alternatively, a lipid-induced signal sequence conformational change may be essential for proper interaction of the secreted protein with a proteinaceous transport machinery. In this section, we review studies which demonstrate that these peptides indeed adopt various conformations depending on the nature of their interaction with lipid.

We examined the conformation of the WT LamB signal peptide bound to phospholipid monolayers by CD and IR spectroscopy (Briggs *et al.*, 1986). If monolayers are initially held at high surface pressure, peptide insertion into the hydrocarbon region of the bilayer is prevented. This condition was employed to examine peptide conformation upon binding to the monolayer surface. The peptide adopts solely $\beta$ structure when localized at the monolayer surface. However, at low initial pressures, where insertion is allowed, a mixture of secondary structure was observed. A relatively high proportion of $\alpha$ helix was evident, which is consistent with the expected conformation of the hydrophobic core region when inserted into the apolar monolayer interior.

This result is supported by studies on peptide conformation when bound to bilayer vesicles, where approximately 60% α helix was observed (McKnight *et al.*, 1989). If residues 10–25 are considered to adopt a helix, as supported by the previously discussed 2D-NMR experiments (Bruch *et al.*, 1989), approximately 30% β structure would be obtained if residues 1–8 adopt an extended (β-like) conformation. This expectation is consistent with the CD and IR data. These results suggest a model in which the hydrophobic core region inserts into the hydrocarbon region as an α helix, and the positively charged N-terminal region remains electrostatically bound to the lipid surface where it occupies a β sheet-like conformation.

We recently determined the orientation of the peptide in each conformational state in monolayers using polarized IR spectroscopy (Cornell *et al.*, 1989). Under conditions where insertion is permitted, the helical region of the peptide is collinear with the phospholipid acyl chains. Also, as expected, the β structure was observed to lie parallel to the monolayer surface. These results strongly support the conclusion that the hydrophobic core region of the peptide inserts into and spans the membrane.

This model for peptide orientation upon monolayer binding is consistent with experiments on the PhoE signal peptide (Batenburg *et al.*, 1988b). These authors determined the molecular area of peptide bound to monolayers. In soyPC monolayers, the molecular area calculations supported a model in which the peptide assumes a looped orientation, with both termini facing the surface. However, with negatively charged cardiolipin monolayers, a time-dependent conformational change was observed during which the peptide reoriented to eside predominantly collinear to the lipid acyl chain region, most likely with the N-terminal region facing the water.

It is of interest to contrast these results on peptides corresponding to export signal sequences with those of Tamm (1986) on a synthetic mitochondrial presequence. This peptide interacted with a phospholipid monolayer with its helical axis parallel to the plane of the monolayer. However, mitochondrial presequences exhibit side-to-side amphiphilicity, rather than the end-to-end amphiphilicity of the bacterial signal peptides. Thus, insertion of the helical axis of this peptide through the lipid hydrocarbon region should be less favorable.

## Influence of the Mature Region of Signal Peptide Properties

We recently found that the LamB signal peptide can confer its affinity for a lipid bilayer on a passenger corresponding to the first 28 residues of the mature LamB protein (McKnight, 1990; McKnight, Stradley, and Gierasch, unpublished). The 53-residue-long peptide shows the same interactions as the

signal peptide alone with membranes, and the two regions (signal sequence and mature) show conformational independence of each other in lipid vesicles by CD. These results argue (1) that the signal sequence and adjacent mature region act as separate conformational domains; (2) that the signal sequence can provide a driving force to cause interaction of its attached mature segment with a membrane; and (3) that the signal peptide is "dominant" over its adjacent mature region. Experiments are under way to determine the topology of the signal sequence plus mature region when inserted in bilayer vesicles. Again, these studies support a functional importance of the highly favorable, spontaneous insertion of the signal sequence into membranes, most likely in a step in protein export wherein the signal peptide is called upon to initiate membrane association of the N-terminal portion of the nascent chain.

## Involvement of Lipids in Protein Translocation

As discussed in previous sections, several models envision protein translocation occurring directly via the lipid phase. The major argument raised against this mechanism is the difficulty of overcoming the energy barrier associated with passage of a large protein, containing many hydrophilic residues, through the hydrophobic lipid acyl chain region. However, several studies have demonstrated protein passage across membranes in reconstituted model systems. Protein precursors were demonstrated to insert into lipid vesicles containing signal peptidase (Ohno-Iwashita and Wickner, 1983) and even into vesicles with no additional protein components (Geller and Wickner, 1985; Tien *et al.*, 1989). The studies presented above indicate that there is a close correlation between signal sequence *in vivo* activity and the ability of signal peptides to interact with and perturb the acyl chain region of lipid. Perturbation of lipid structure may alter the energetics associated with protein solubility and transport. In this section, we will examine studies concerning the effects of peptide binding on lipid structure, and summarize studies concerning the involvement of specific lipids in protein translocation.

Negatively charged lipids have been implicated in protein translocation by a limited number of studies. de Vrije *et al.* (1988) showed that translocation was inhibited in *E. coli* when phosphatidylglycerol (PG) biosynthesis was inhibited. This result was obtained *in vivo* as well as in cell-free translocation systems, and complements studies discussed above which demonstrated preferential binding to negatively charged lipids. However, it is unclear whether the observed dependence of translocation on PG is due to a specific interaction between this lipid and the signal sequence. It is also quite

possible that alteration in bulk membrane properties, arising from the dramatic decrease in surface charge, renders the membrane less competent for translocation. Integral membrane proteins involved in translocation as well as lipid structural properties could be adversely affected.

A highly speculative model for the involvement of acid phospholipids in bacterial membrane translocation has been proposed by Nesmayanova (1982) and refined recently by Nesmeyanova and Bogdanov (1989). In this treatment, the signal sequence electrostatically binds to the negatively charged lipid cardiolipin, and the hydrophobic core region inserts into the membrane. Subsequently, the peptide–lipid interaction is proposed to catalyze the conversion of cardiolipin to PG. This electrostatically bound PG is proposed to carry the signal sequence to the opposite side of the membrane, via rapid transmembrane movement. Much future work is necessary to test this model, however, and nothing is known regarding the effect of signal sequence insertion on phospholipid metabolism. Likewise, there are no data which support the hypothesis that lipid transmembrane movement is accelerated by signal sequence insertion.

Nonbilayer lipid structures have also been postulated to mediate protein translocation via the lipid phase (de Kruijff *et al.*, 1985), and there are data from model systems which implicate signal peptides in the formation of these structures. de Kruijff and co-workers have demonstrated that WT PhoE signal peptide has the capacity to induce formation of nonbilayer structures in certain lipid systems (Batenburg *et al.*, 1988; Killian *et al.*, 1990). Freeze-etch electron microscopy and $^{31}$P NMR spectroscopy supported the conclusion that this peptide induced the formation of type II hexagonal lipid phases. Transient, local formation of this lipid structure could result in aqueous pores in membranes, through which proteins can efficiently translocate. Also, it has been proposed that protein translocation occurring in conjunction with lipid transmembrane movement is catalyzed by the formation of these nonbilayer structures. However, these postulates remain very speculative at this point.

The tendency of lipid systems to adopt type II nonbilayer phases is related to structural parameters which may affect protein solubility in the membrane. Gruner (1985) presented a theoretical model whereby the propensity for formation of nonbilayer structures is dependent on the spontaneous radius of curvature of the lipid surface, which is a measure of the surface free energy. Predictions derived from this model describe experimental data quite accurately (Siegel *et al.*, 1989; Gruner *et al.*, 1988). Induction of phase II structures by signal peptides in model systems suggests that signal sequence binding could change the surface energy of secretory membranes. Little is known regarding the properties of lipid systems which are perturbed from their typical surface curvature values. Locally altered lipid properties, such as local dielectric

constant (see below), could lead to enhanced protein permeability without altering the bilayer arrangement of the lipid phase. This proposed mechanism, more than formation of local nonbilayer phases, can accommodate the structural requirements involved in preservation of membrane integrity.

Even without invoking nonbilayer phases or surface curvature changes, there are various ways signal sequence binding could perturb lipid structure in functionally significant ways. For instance, the calculated rate of protein association with phospholipid bilayers has been shown to be critically related to bilayer dielectric constant. Roseman (1988) demonstrated that association of helical hairpin loops with bilayers becomes much more favorable when the dielectric gradient in membranes is properly considered, as opposed to treating the hydrocarbon region of the bilayer as an alkane phase. He showed that a rate constant derived using a dielectric gradient in the bilayer described experimental data (Leto and Holloway, 1979) better than one that treats the bilayer hydrocarbon region as an alkane phase. Since energetics and association rate constants are logarithmically related, relatively small changes in association free energy result in large changes in binding rates. Alteration of bilayer dielectric properties upon insertion of signal sequences may thus facilitate subsequent protein insertion. Nothing is known regarding the effect of signal peptide binding upon bilayer dielectric constant. However, McKnight (1990) showed that vesicle binding of the LamB signal peptide enhanced permeability of 6-carboxyfluoroscein, which may reflect an increased water content in the bilayer acyl chain region. Also, Jacobs and White (1989) have demonstrated that binding of tripeptides of the form Ala-X-Ala-O-*tert*-butyl causes increased bilayer water content. Thus, it is reasonable to assume that signal peptide binding increases bilayer dielectric constant and may significantly affect the capacity of a lipid bilayer to accommodate protein translocation.

## Inhibition of *in Vitro* Translocation by Synthetic Signal Sequence

An interesting bridge between the biophysical characterization described in the preceding sections and biochemical analyses of protein export is provided by *in vitro* translocation experiments in the presence of added synthetic signal peptides. We have shown that the LamB signal peptides can inhibit translocation of precursor proteins (pre-OmpA and pre-alkaline phosphatase) across native *E. coli* membranes (Chen *et al.*, 1987). Previously, similar observations were made in eukaryotic systems where preproparathyroid signal peptide (Majzoub *et al.*, 1980) or a consensus signal peptide (Austen *et al.*, 1984) inhibited translocation across microsomal membranes at peptide concentrations of 5–10 $\mu$M. It was particularly persuasive in our

study that the export-defective LamB deletion mutant (Fig. 2) inhibited only weakly, followed by the two revertant sequences, which inhibited to extents correlating with their respective abilities to export the protein *in vivo*, and the wild type, which was most strongly inhibitory. The concentrations required for inhibition (ca. 1 $\mu$M), however, were far greater than the radiochemical amounts of precursor proteins and were in fact approximately the concentrations at which the peptides bound to lipid monolayers (McKnight *et al.*, 1989). Hence, the mechanism of inhibition could either be via interactions with the lipids of the membrane or via binding to a proteinaceous signal sequence receptor in the membrane or cytosol. We have explored these alternatives by synthesis of a mirror-image signal peptide to the LamB wild type (A. Sgrignoli, L. Chen, P. C. Tai, and L. Gierasch, unpublished results). The all-D signal peptide was expected to interact with the lipids analogously to the all-L, but would not bind as well to a proteinaceous receptor. We found that the two precursor proteins were differentially sensitive to the two peptides: Translocation of pre-alkaline phosphatase was inhibited to the same extent by LamB or its mirror image. Translocation of pre-OmpA was inhibited more by the all-L peptide. We interpret these results as indicating that different mechanistic steps may be critical for different precursors, and that lipid interactions are likely to be more directly involved in the case of translocation of pre-alkaline phosphatase. Future work along these lines will exploit the synthetic peptides as probes of specific signal sequence binding interactions with proteinaceous components of the protein export apparatus.

## Implications of Signal Peptide Studies

Although conclusions from studies of synthetic signal peptides must be cautiously extrapolated to the *in vivo* roles of signal sequences, the properties that have been described for the isolated peptides must influence the interactions and folding of signal sequences in any situation. Most significant are the results which demonstrate that peptides corresponding to functional signal sequences show clear biases toward stable helix in the hydrophobic core, high affinities for lipid, abilities to insert into the acyl chain region of bilayers, and capacity to inhibit *in vitro* protein translocation in *E. coli* membrane vesicles. Either the components of the protein export pathway have evolved to exploit the same properties of signal sequences that enable them to bind lipids or there are direct lipid interactions of these sequences *in vivo*. It is possible that the current vintage of signal sequences reflects earlier selection pressures at a time when protein secretion occurred via a mechanism including direct lipid interactions, but that present-day export is always handled by proteinaceous species. We still favor a model in which the

signal sequence, subsequent to its release from an SRP-like cytoplasmic factor, inserts into the membrane. It is then constrained to two-dimensional diffusion, and its perturbation of the lipid can assist in recruitment of a protein channel or in allowing the nascent chain to penetrate the lipid bilayer.

Work on the influence of signal peptides on lipid properties suggests that this idea of a functional ramification of peptide-mediated lipid reorganization is reasonable. Too little is known at present, and the results are confined to a few peptides, but it is definitely worth pursuing in greater detail the effects of signal peptide insertion on lipids.

Results discussed in this review raise several questions about possible roles of lipids, and together with developments in other areas of cell biology (Berridge and Irvine, 1989; Schafer *et al.*, 1989) underline the importance of considering lipids as active players in the activities of a cell. We fully expect that the detailed mechanism of protein export will involve the dynamic participation of the signal sequence on the nascent chain, phospholipids of the membrane, and proteinaceous components of the export apparatus.

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