Membrane Interaction and Conformational Properties of the Putative Fusion Peptide of PH-30, a Protein Active in Sperm–Egg Fusion†

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ABSTRACT: A peptide representing the putative fusion domain of PH-30, a sperm surface protein involved in sperm–egg fusion, was synthesized, and its interaction with model lipid membranes was characterized by biophysical methods. While the peptide binds to the vesicles composed of both neutral and acidic lipids, the apparent affinity is significantly higher for the latter lipid class. The intervesicular lipid mixing assay suggests that the synthetic peptide is able to induce fusion of large unilamellar vesicles. Circular dichroism and Fourier-transform infrared spectroscopy show that while in an aqueous buffer the peptide exists in an essentially unordered conformation, binding to the membranes results in a conformational transition to a β-structure. These data indicate that the fragment identified on the α-subunit of PH-30 as a putative fusion peptide is indeed a good candidate for this role. However, in contrast to what has been proposed for some viral fusion peptides, the PH-30 fusion domain is highly unlikely to act as an insertional "sided" helix.

Membrane fusion is a widespread event that plays a crucial role in important physiological processes such as endocytosis, exocytosis, secretion, or fertilization. Fusion events are also associated with virus-induced pathogenesis of a number of disease states. Studies on the molecular mechanism of viral fusion have allowed identification and characterization of specific fusion proteins which, when reconstituted into liposomes, are sufficient to confer the fusion activity [for recent reviews, see Marsh and Helenius (1989), Wilschut and Hoekstra (1991), White (1992), and Zimmerberg et al. (1993)]. One of the main characteristics of most viral fusion proteins is the presence of relatively hydrophobic amino acid sequences, often referred to as "fusion peptides". The biophysical properties of such fusion peptides, especially the mechanisms of their membrane action, are the subject of intense research (Murata et al., 1987; Wharton et al., 1988; Lear & DeGrado, 1987; Rafalski et al., 1990, 1991; Yeagle et al., 1991; Düzgünes & Shavnin, 1992; Martin et al., 1992).

In a recent report, Blobel et al. (1992) deduced the amino acid sequence of PH-30, a sperm surface protein involved in sperm–egg fusion. The sequence data provided strong support for the earlier hypothesis (Blobel et al., 1990) that PH-30 shares many common characteristics with viral fusion proteins. This, in turn, led to an intriguing suggestion that fusion events as disparate as the fertilization of ovaries and penetration of enveloped viruses into host cells may share common mechanisms and involve highly conserved motifs in the family of membrane proteins (Blobel et al., 1992; White, 1992). In this context, particular interest exists in identifying and characterizing a fusion peptide in the sperm surface protein. The sequence recently suggested as a likely candidate for this role comprises residues 89–111 of the α-subunit of PH-30 (Blobel et al., 1992). Analogous to the fusion peptides of viral proteins, this sequence has been modeled as a "sided" α-helix (Blobel et al., 1992; White, 1992). However, the predictions regarding the fusogenic activity and conformational properties of the putative fusion peptide in PH-30 have not yet been tested experimentally.

In the present study, we have synthesized a peptide comprising the putative fusion sequence in PH-30. The fragment has the sequence Ac-K-L-I-C-T-G-I-S-S-I-P-P-I-R-A-L-F-A-A-I-Q-I-P-H-NH_2 (region 89–112 of the α-subunit of the protein). The results indicate that this peptide has a high affinity for membrane lipids and is indeed fusogenic. However, in contradiction to an earlier hypothesis, the preferred conformation of the peptide in a lipid environment appears to be not an α-helix but a β-sheet structure.

MATERIALS AND METHODS

Peptide Synthesis and Purification. The peptide PH-30α-(89–112) and its fluorescent analog [Trp^{95}]PH-30α(89–112) were synthesized in a MilliGen PepSynthetizer 9050 Plus, using Fmoc-protected amino acids and a Rink amide continuous-flow solid support resin (Rapp Polymere, Tübingen, Germany) (Fields & Noble, 1990; Rink, 1987). The side chains were protected by a Boc group (Lys, Trp), a trityl group (Gln, His), a *tert*-butyl group (Thr, Ser), and a 2,2,5,7,8-pentamethylchroman-6-sulfonyl group (Arg). Coupling was carried out with in situ activation of 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU), *N*-hydroxybenzotriazole, and *N*-methylmorpholine according to Knorr et al. (1989). The product was cleaved from the resin with 95% trifluoroacetic acid (TFA) for 2 h at room temperature using phenol and thioanisole as scavengers. The crude material was purified by HPLC on a reversed-phase C18 Bio-Rad Hi-Pore RP-318 column with 0.1% TFA/H_2O–0.1% TFA/CH_3CN gradient. The final product was identified by FAB/MS and ion spray mass spectroscopy. The recovery of the correct product was very low, largely due to the low yield of coupling of the second proline in the ...I-P-P-I... sequence and the difficulties encountered in the purification step.

Vesicle Preparation. All phospholipids were obtained from Avanti Polar Lipids, and cholesterol was from Sigma Chemical

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Co. Large unilamellar vesicles (LUV)[1] were prepared by the method of Hope et al. (1985). Dried lipid films were dispersed in buffer and freeze-thawed 10 times by using liquid nitrogen/lukewarm water cycles. This was followed by 10 cycles of extrusion through two stacked 100-nm pore size polycarbonate filters (Nucleopore Corp.) in a pressure extruder (Lipex Biomembranes, Inc., Vancouver, Canada). Small unilamellar vesicles (SUV) were prepared by sonication of lipid dispersions in an ice bath for about 15 min using a probe-type sonifier. Metal debris from the titanium tip was removed by centrifugation for 20 min at 16000g. Unless indicated otherwise, the composition of the buffer was 20 mM MES, 100 mM NaCl, and 1 mM DTT, pH 6.5.

Binding Experiments. Peptide–membrane binding experiments were performed with [Trp^{95}]PH-30α(89–112) by following changes in the fluorescence spectra of the tryptophan residue of the peptide upon its incubation with increasing amounts of lipid vesicles. For this purpose, 2-μL aliquots of the stock solution of the peptide in methanol (0.5 mM) were added to 200 μL of the buffer containing increasing amounts of SUV or LUV. Prior to fluorescence measurements, samples were incubated at room temperature for 15 min while being shaken. Fluorescence spectra were recorded at 20 °C in 3-mm path-length quartz cell, with an excitation wavelength of 285 nm, using a SLM-8000 spectrofluorometer. The fluorescence titration curves were analyzed as described previously (Surewicz & Epand, 1984) to obtain the apparent affinity constant K_d/n, where K_d is the dissociation constant and n represents the number of binding sites per lipid. The K_d/n parameter has been shown to provide a reliable measure of membrane affinity for the ligand (Bashford et al., 1979).

Lipid Mixing Assay. Peptide-induced fusion of LUV was measured by the fluorescence resonance energy transfer between NBD-PE and Rh-PE (Struck et al., 1981) essentially as described previously (Surewicz et al., 1985). In brief, fusion was initiated by addition of the peptide in methanol or dimethyl sulfoxide to a mixture of LUV labeled with both NBD-PE and Rh-PE at 1 mol % each and unlabeled LUV at a molar ratio of 1:6. The final concentration of organic solvent was kept below 1%. The mixture was incubated while being shaken for 30 min at room temperature, and then fluorescence spectra were recorded using excitation at 450 nm. The percentage of fusion (lipid intermixing) was determined from the extent of resonance energy transfer in a given sample and the appropriate calibration curve (Surewicz et al., 1985).

Circular Dichroism. Spectra were obtained on a JASCO J-600 spectropolarimeter at room temperature using a 0.5-cm cylindrical cell and peptide concentrations of 15 μM. Samples for CD spectroscopy were prepared by adding small aliquots of the stock peptide solution in methanol to the buffer (0.7 mM MES, 100 mM NaF, 0.5 mM DTT, pH 6.5) or to the buffer containing SUV. The final concentration of methanol was below 1%.

FT-IR Spectroscopy. Samples for infrared spectroscopy were prepared by mixing 10 μL of a 4 mg/mL peptide solution in methanol with 1 mL of the suspension of LUV (approximately 1 mg of lipid/mL) in deuterated buffer. The mixture was incubated for 30 min at room temperature, and the lipid–peptide complexes were concentrated to a final volume of approximately 40 μL using the Millipore Ultrafree-MC centrifugal filtration system. Spectra were recorded on a Bio-Rad FTS-40A instrument, with samples assembled in a 50-μm path-length calcium fluoride cell. Typically, 264 interferograms were coadded and Fourier-transformed to give a resolution of 2 cm^{-1}.

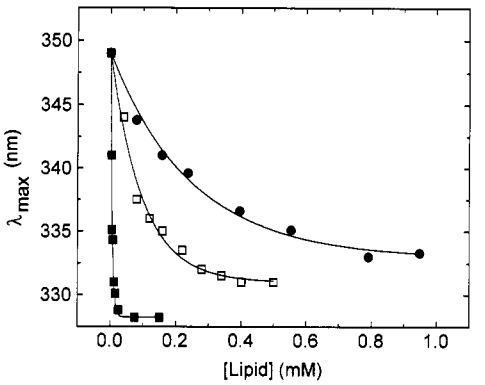

FIGURE 1: Position of the maximum in fluorescence spectra of [Trp^{95}]-PH-30α(89–112) in the presence of small unilamellar vesicles as a function of lipid concentration: (■) POPG; (○) POPC; (□) POPC/POPG/PE (1:1:1).

RESULTS

Membrane Binding. In order to study the interaction of the putative PH-30 fusion peptide with lipid vesicles by fluorescence spectroscopy, we have prepared the peptide analog in which Ile at position 95 was replaced by Trp. It has been verified that the properties of the fluorescent peptide are essentially identical to those of the parent molecule, both with respect to the conformation as well as the ability to induce vesicle fusion (see below).

In aqueous solution, [Trp^{95}]PH-30α(89–112) gives a fluorescence spectrum with an emission maximum at 349 nm. This indicates a highly polar environment of the tryptophan residue and is typical for short- or medium-size peptides in a largely unordered conformation (Lear & DeGrado, 1987; Surewicz & Epand, 1984). In the presence of phospholipid vesicles, the maximum emission is shifted to shorter wavelengths, and there is an enhancement of the fluorescence intensity. The observed blue shift reflects the decreased polarity of the tryptophan environment and is indicative of at least partial immersion of the indole ring in the membrane. Figure 1 illustrates the change in fluorescence maximum when the peptide is titrated with increasing concentrations of SUV of various lipid compositions. The peptide interacts with both acidic POPG and zwitterionic POPC, although the blue shift is significantly more pronounced and the binding is much stronger in the case of POPG than POPC. Quantitative analysis of the binding curves of Figure 1 gives apparent dissociation constants, K_d/n, of 4×10^{-6} M, 2.3×10^{-4} M, and 1×10^{-4} M for the peptide binding to the vesicles of POPG, POPC, and POPC/POPG/PE (1:1:1), respectively. Membranes of the last composition were used in the functional fusion assay.

POPG was used throughout these studies as a model acidic phospholipid. Unlike the physiologically more relevant phosphatidylserine, phosphatidylglycerol does not interfere with the peptide amide I infrared band or far-UV CD spectrum. This lipid therefore provides a suitable model for exploring correlations between conformational properties of the membrane-bound peptide and its lipid affinity and fusogenic

[1] Abbreviations: LUV, large unilamellar vesicles; SUV, small unilamellar vesicles; MES, 2-(N-morpholino)ethanesulfonic acid; DTT, dithiothreitol; NBD-PE, N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)phosphatidylethanolamine; Rh-PE, N-(lissamine rhodamine B sulfonyl)phosphatidylethanolamine; CD, circular dichroism; FT-IR, Fourier-transform infrared; POPG, 1-palmitoyl-2-oleoylphosphatidylglycerol; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; POPS, 1-palmitoyl-2-oleoylphosphatidylserine; PE, egg phosphatidylethanolamine.

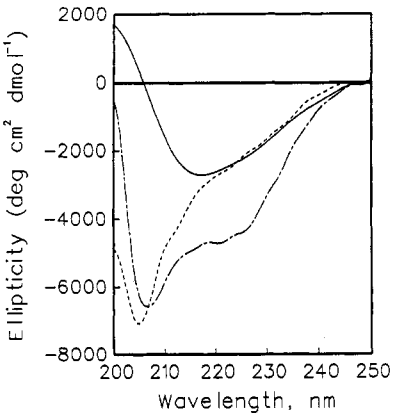

FIGURE 2: Far-ultraviolet circular dichroism spectra of PH-30α-(89–112) in an aqueous buffer (- - -) and methanol (– – –), and in the presence of POPG vesicles at a lipid to peptide molar ratio of 60:1 (—).

activity. It should be noted, however, that strong peptide binding found for POPG seems to represent the property of the whole class of acidic lipids as a fluorescence binding curve very similar to that shown for POPG was obtained for SUV prepared from POPS. The apparent dissociation constant, K_d/n, for the POPS vesicles is 3.4×10^{-6} M. Furthermore, a blue shift in the peptide fluorescence spectrum similar to that shown in Figure 1 was also observed when SUV were replaced with LUV, although only limited data are available for the latter system and fitting of the titration curves is less reliable due to much higher light scattering of LUV. The estimated value of K_d/n for the peptide binding to LUV prepared from POPG is 4.4×10^{-6} M.

Conformational Properties. The circular dichroism spectrum of PH-30α(89–112) in aqueous buffer (Figure 2) is strongly suggestive of a largely unordered conformation, although the minimum at 204 nm is slightly red-shifted compared to the spectra of typical random-coiled structures (Yang et al., 1986). While this slight red shift may be indicative of a small proportion of β-structure, the very low intensity at 222 nm clearly argues against significant α-helical content. The spectrum of the peptide in methanol, a strong promoter of α-helix, has two minima: the stronger one at 206 nm and the weaker one around 220 nm. These general features are indicative of a partially α-helical structure, although the relatively low value of negative ellipticity at 220 nm (only 4700 deg cm^2 dmol^{-1}) suggests that the proportion of helix is rather low. Indeed, quantitative evaluation of the spectrum (Neugebauer et al., 1992) yields only 17% α-helix. The CD spectrum of the peptide bound to the POPG vesicles is remarkably different: it has a minimum at 215 nm and the crossover at 205 nm. These features are highly characteristic of a β-structure (Yang et al., 1986). Attempts to obtain the spectrum of PH-30α(89–112) bound to POPC vesicles were unsucessful. Saturation binding to the latter lipid requires a much higher vesicle concentration, and under such conditions the combined effects of light scattering and lipid absorption precluded reliable circular dichroism measurements.

An independent verification of the conformation of the peptide in a membrane environment was obtained by FT-IR spectroscopy. This method is of particular value in studying membrane-bound proteins since, in contrast to the CD spectra, the infrared measurements are free from potential artifacts due to light scattering of the vesicles. The FT-IR spectra of PH-30α(89–112) associated with POPC vesicles, POPG vesicles, or vesicles composed of an equimolar mixture of POPG, POPC, and PE are very similar. The conformation-sensitive amide I region of these spectra is clearly dominated by a strong band around 1624 cm^{-1} (Figure 3). This band is highly characteristic of a β-sheet structure (Surewicz & Mantsch, 1988). The weak and broad feature between approximately 1640 and 1680 cm^{-1} may contain contributions from unordered structure, turns, and (possibly) α-helix. Since the signal-to-noise ratio of the spectra obtained in this study was rather poor (as a result of the relatively low peptide concentrations used), no attempt was made to resolve these minor contributions by Fourier self-deconvolution. Infrared studies of PH-30α(89–112) in aqueous solution were precluded by insufficient solubility of the peptide.

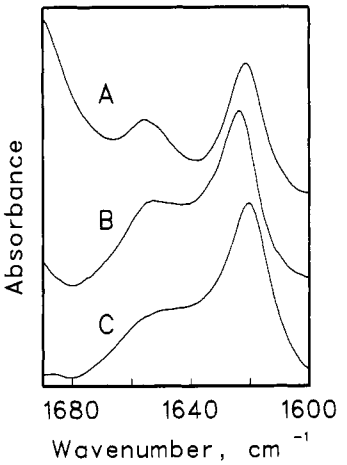

FIGURE 3: Infrared spectra in the amide I region of PH-30α(89–112) associated with the vesicles of POPC at a lipid to peptide molar ratio of 200:1 (A) or POPG at a lipid to peptide molar ratio of 60:1 (B) or an equimolar mixture of POPC, POPG, and PE at a lipid to peptide molar ratio of 100:1 (C).

Table 1: Peptide-Induced Fusion of POPC/POPG/PE (1:1:1) Vesicles

[peptide], μM	% fusion[a]	[peptide], μM	% fusion[a]
0	0	20	20
10	14	30	51

[a] Percent fusion is defined as a percentage of a maximal possible lipid intermixing between large unilamellar vesicles after 30-min incubation with the peptide. The lipid concentration was 0.4 mM.

Lipid Mixing. The fusogenic potential of PH-30α(89–112) was assessed by measuring the ability of the peptide to induce phospholipid mixing between the population of vesicles containing the fluorescent probes NBD-PE and Rh-PE and the label-free vesicles (Struck et al., 1981). The results summarized in Table 1 indicate that addition of the peptide to POPC/POPG/PE LUV results in an effective intervesicular lipid mixing. Although only limited data are available, the effect is clearly concentration dependent and increases with the increasing amount of peptide added. Essentially identical results to those in Table 1 were obtained when vesicles of the same phospholipid composition but containing 25 mol % of cholesterol were used (data not shown).

DISCUSSION

A common feature of most viral fusion proteins is the presence of fusion domains, also known as fusion peptides. These peptides are stretches of 16–26 amino acids of a relatively high average hydrophobicity index and are located either internally or at the amino terminus of the membrane-anchored subunit. Fusion peptides are believed to play an important functional role by facilitating the hydrophobic interaction

between the virus and the target membrane and promoting the actual fusion reaction. The involvement of the amino-terminal fusion domains of a number of viral envelope glycoproteins in mediating the fusion event has been indicated by site-directed mutagenesis and hydrophobic photolabeling experiments (Gething et al., 1986; Novick & Hoekstra, 1988; Bosch et al., 1989; Horth et al., 1991). Furthermore, synthetic sequences representing fusion peptides in the above proteins have been shown to interact with model membranes and induce fusion of phospholipid vesicles (Murata et al., 1987; Wharton et al., 1988; Lear & DeGrado, 1987; Rafalski et al., 1990, 1991; Düzgünes & Shavnin, 1992; Martin et al., 1992).

It has been recently proposed that PH-30, a multisubunit integral membrane protein involved in sperm–oocyte fusion, shares important structural and functional similarities with viral fusion proteins (Blobel et al., 1992). One of these similarities is the presence of a putative fusion peptide which, based on sequence comparison and modeling exercises, has been identified as a 23 amino acid segment (residues 89–111) on the α-subunit of PH-30. A notable feature of the β-subunit is the presence of a disintegrin-like domain. Further progress in elucidating molecular mechanisms of egg–sperm fusion seems to be critically dependent on the detailed characterization of PH-30 and, especially, on the understanding of the structure–function relationships of the individual functional domains of the protein.

The goal of these studies was to explore the biophysical and conformational properties of the putative fusion domain of PH-30. Studies with synthetic peptides representing putative fusion domains of viral proteins have provided useful hints regarding the molecular mechanisms of viral fusion, especially in relation to the early steps of the fusion process. It should be pointed out, however, that the strategies based on the use of synthetic peptides and model membranes have obvious limitations since many features of the intact biological system are not present in simple model systems. The uses and limitations of peptide models for viral fusion have been recently discussed by Epand et al. (1992). The arguments presented by these authors apply equally well to peptide models for other types of biological fusion events.

The present data demonstrate that, like many viral fusion peptides, the synthetic peptide representing the putative fusion sequence in PH-30 has the ability to interact with phospholipid bilayers. A relatively strong association can be detected with the vesicles containing POPC as a sole lipid component. The apparent binding affinity is further increased (by a factor of approximately 50) when zwitterionic POPC is replaced with acidic phospholipids. The binding experiments with model vesicles thus suggest that the interaction of the peptide with a target plasma membrane containing a variety of lipid classes is likely to be governed by a combination of hydrophobic and electrostatic effects.

Binding of the peptide to the vesicles is followed by intervesicular lipid mixing, as assessed by the resonance energy transfer assay. The efficiency of PH-30α(89–112)-induced lipid mixing is generally comparable to that reported for the viral fusion peptides (Murata et al., 1987; Lear & DeGrado, 1987; Martin et al., 1992). Intervesicular lipid mixing is usually considered as indicative of vesicle fusion (Murata et al., 1987; Wharton et al., 1988; Lear & DeGrado, 1987; Martin et al., 1992; Struck et al., 1981). Such an interpretation, however, is not always error-proof as lipid mixing may conceivably occur by other mechanisms [e.g., exchange of labeled lipid molecules between aggregated vesicles or hemifusion (Düzgünes et al., 1987)]. Further experiments employing techniques such as electron microscopy or aqueous content mixing assays are needed to independently verify and better characterize the fusogenic action of PH-30α(89–112). The progress of these studies is somewhat impeded by difficulties encountered in obtaining larger quantities of the purified peptide. Nevertheless, with all these limitations, the combined results of the binding experiments and lipid mixing studies provide the first experimental evidence that the segment on the α-subunit of PH-30, previously identified as a putative fusion peptide, is indeed a good candidate for this role.

The most advanced hypothesis regarding the role of fusion peptides in the mechanism of viral fusion postulates that these peptides act as sided insertional helices (Harter et al., 1989; Brasseur et al., 1990; Horth et al., 1991). Such helices are characterized by an asymmetric distribution of the bulky hydrophobic residues. The more hydrophobic side of the helix is believed to insert into the membrane with an oblique orientation with respect to the lipid–water interface, leading to a local disorganization of the bilayer structure. In analogy with the viral fusion peptides, the putative fusion peptide of PH-30 has been modeled as a sided insertional helix (Blobel et al., 1992; White, 1992). However, the presence of a number of strong helix-breaking residues (glycine, two sequential serines, two sequential prolines) raises serious questions regarding the ability of the latter peptide to fold into a helical structure. These doubts are reinforced by the spectroscopic data. The peptide is essentially unordered in water and becomes only slightly helical in strongly helix-promoting solvents such as methanol. Most significantly, association of PH-30α(89–112) with lipid vesicles clearly does not promote transition to a helical structure. In contrast, the CD and FT-IR measurements indicate that the preferred conformation of the peptide in a lipid environment is a β-sheet structure.

While the most common conformation of membrane-associated peptides is α-helical, the lipid-induced transition to a β-structure is not without precedent. For example, signal peptides have been shown to interconvert between β-structure, when absorbed onto the surface of the lipid monolayer, and α-helical conformation, when inserted into the hydrophobic interior of the lipid phase (Briggs et al., 1986). The precise membrane location and orientation of the PH-30 fusion peptide are at present unknown. However, a strong lipid-induced blue shift in the fluorescence spectrum of the tryptophan-containing analog suggests that the peptide–membrane interaction is not simply a surface absorption, and at least some portion of the peptide chain is immersed in the hydrophobic interior of the lipid bilayer.

Although there is substantial experimental evidence that certain viral fusion peptides can indeed adopt α-helical conformation when placed in the lipid environment, important reservations regarding the sided insertional helix hypothesis as a universal mechanism for induction of membrane fusion have been recently expressed by Gallaher et al. (1992). Inconsistent with the above hypothesis are also recent CD data for the measles fusion peptide (Epand et al., 1992). The present spectroscopic experiments with the PH-30 fusion peptide further reinforce these reservations. Clearly, some peptides corresponding to the putative fusion domains favor conformations other than α-helix. Further studies are needed to explore the mechanism by which the nonhelical structures, and the β-structure in particular, can catalyze membrane fusion events.

REFERENCES

Bashford, C. L., Chance, B., Smith, J. C., & Yoshida, T. (1979) *Biophys J. 25*, 63– 85.

Blobel, C. P., Myles, D. G., Primakoff, P., & White, J. M. (1990) *J. Cell Biol. 111*, 69–78.
Blobel, C. P., Wolfsberg, T. G., Turck, C. W., Myles, D. G., Primakoff, P., & White, J. M. (1992) *Nature 356*, 248–252.
Bosch, M. L., Earl, P. L., Fargnoli, K., Picciafuoco, S., Giombini, F., Wong-Staal, F., & Franchini, G. (1989) *Science 244*, 694–697.
Brasseur, R., Vandenbranden, M., Cornet, B., Burny, A., & Ruysschaert, J.-M. (1990) *Biochim. Biophys. Acta 1029*, 267–273.
Briggs, M. S., Cornell, D. G., Dluhy, R. A., & Gierasch, L. M. (1986) *Science 233*, 206–208.
Düzgünes, N., & Shavnin, S. A. (1992) *J. Membr. Biol. 128*, 71–80.
Düzgünes, N., Allen, T. M., Fedor, J., & Papahadjopoulos, D. (1987) *Biochemistry 26*, 8435–8442.
Epand, R. M., Cheetham, J. J., Epand, R. F., Yeagle, P. L., Richardson, C. D., Rockwell, A., & DeGrado, W. F. (1992) *Biopolymers 32*, 309–314.
Fields, G. B., & Noble, R. L. (1990) *Int. J. Pept. Protein Res. 35*, 161–214.
Gallaher, W. R., Segrest, J. P., & Hunter, E. (1992) *Cell 70*, 531–532.
Gething, M.-J., Doms, R. W., York, D., & White, J. (1986) *J. Cell Biol. 102*, 11–23.
Harter, C., James, P., Bächi, T., Semenza, G., & Brunner, J. (1989) *J. Biol. Chem. 264*, 6459–6464.
Hope, M. J., Bally, M. B., Webb, G., & Cullis, P. R. (1985) *Biochim. Biophys. Acta 812*, 55–65.
Horth, M., Lambrecht, B., Chuah, M., Khim, L., Bex, F., Thiriart, C., Ruysschaert, J.-M., Burny, A., & Brasseur, R. (1991) *EMBO J. 10*, 2747–2755.
Knorr, R., Trzeciak, A., Bannawarth, W., & Gillessen, D. (1989) *Tetrahedron Lett. 30*, 1927–1930.
Lear, J. D., & DeGrado, W. F. (1987) *J. Biol. Chem. 262*, 6500–6505.
Marsh, M., & Helenius, A. (1989) *Adv. Virus Res. 36*, 107–151.
Martin, I., Defrise-Quertain, F., Nielsen, N. M., Saermark, T., Burny, A., Brasseur, R., Vandenbranden, M., & Ruysschaert, J. M. (1992) in *Membrane Interactions of HIV: Implications for Pathogenesis and Therapy of AIDS* (Aloia, R. C., & Curtain, C. C., Eds.) pp 365–376, Wiley-Liss, New York.
Murata, M., Sugahara, Y., Takahashi, S., & Ohnishi, S. (1987) *J. Biochem. 102*, 957–962.
Neugebauer, W., Surewicz, W. K., Gordon, H. L., Somorjai, R. L., Sung, W., & Willick, G. E. (1992) *Biochemistry 31*, 2056–2063.
Novick, S. L., & Hoekstra, D. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 7433–7437.
Rafalski, M., Lear, J. D., & DeGrado, W. F. (1990) *Biochemistry 29*, 7917–7922.
Rafalski, M., Oritz, A., Rockwell, A., van Ginkel, L. C., Lear, J. D., DeGrado, W. F., & Wilschut, J. (1991) *Biochemistry 30*, 10211–10220.
Rink, H. (1987) *Tetrahedron Lett. 28*, 3787–3790.
Struck, D. K., Hoekstra, D., & Pagano, R. E. (1981) *Biochemistry 20*, 4093–4099.
Surewicz, W. K., & Epand, R. M. (1984) *Biochemistry 23*, 6072–6077.
Surewicz, W. K., & Mantsch, H. H. (1988) *Biochim. Biophys. Acta 952*, 115–130.
Surewicz, W. K., Epand, R. M., Vail, W. J., & Moscarello, M. A. (1985) *Biochim. Biophys. Acta 820*, 319–323.
Wharton, S. A., Martin, S. R., Ruigrok, R. W. H., Skehel, J. J., & Wiley, D. C. (1988) *J. Gen. Virol. 69*, 1847–1857.
White, J. M. (1992) *Science 258*, 917–924.
Wilschut, J., & Hoekstra, D. (1991) *Membrane Fusion*, Marcel Dekker, New York.
Yang, J. T., Wu, C. S., & Martinez, H. (1986) *Methods Enzymol. 130*, 208–269.
Yeagle, P. L., Epand, R. M., Richardson, C. D., & Flanagan, T. D. (1991) *Biochim. Biophys. Acta 1065*, 49–53.
Zimmerberg, J., Vogel, S. S., & Chernomordik, L. V. (1993) *Annu. Rev. Biophys. Biomol. Struct. 22*, 433–466.