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ADVANCES IN MEMBRANE FLUIDITY

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Membrane Interactions of HIV

Implications for Pathogenesis and Therapy in AIDS

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Study of the Interaction Between Lipids and the NH_2-Terminal Peptide of Simian Immunodeficiency Virus Fusion Protein

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INTRODUCTION

Virus–host cell fusion is one of the primary events in the infection process induced by enveloped viruses (White, 1990). Evidence suggesting the role of viral envelope glycoprotein in membrane fusion has come from site-directed mutagenesis studies (Gething et al., 1986; Kowalski et al., 1987; Bosch et al., 1989; Freed and Risser, 1990) and the use of antibodies that inhibit fusion without affecting virus binding (Rusche et al., 1988; Linsley et al., 1988; Skinner et al., 1988). Many viral fusion proteins are synthesized as larger precursors and cleaved in two fragments: One anchors in the viral membrane, and the other remains associated with it through disulfide bonds or noncovalent interactions (Stegmann et al., 1989). This endoproteolytic cleavage is required for viral infectivity, since site-directed mutagenesis of the cleavage sequence reduces the efficiency of precursor processing and inhibits syncytium formation (McCune et al., 1988; Freed and Risser, 1990). The surface glycoprotein contains the receptor-binding domain, while the transmembrane protein plays a crucial role in the fusion mechanism.

A striking feature of most, but not all, viral fusion proteins is the presence at the N terminus of the transmembrane protein subunit of a fusion peptide (Gallaher, 1987), generated by cleavage of the precursor glycoprotein, which contains mainly hydrophobic amino acids. The length and the amino acid sequence of this fusion peptide vary between virus families, but is well conserved among various strains of a particular virus. Mutagenesis studies (Gething et al., 1986) and photoaffinity labeling experiments (Harter et al., 1989) have demonstrated the involvement of the amino-terminal fusion domain of the influenza hemagglutinin in the fusion process. Mutations that disrupt the hydrophobicity of the amino terminus of influenza hemagglutinin HA_2 inhibited the fusion process, suggesting that hydrophobicity is a key component of the fusion reaction (Gething et al., 1986). The motif Phe-X-Gly (where X is any

of several amino acids) appears in the fusion domain of several viruses (Gallaher, 1987). Photolabeling of the bromelain-released fragment of the influenza hemagglutinin after interaction with liposomes at low pH has revealed that labeling occurred specifically in the fusogenic domain (Harter et al., 1989), suggesting that this peptide inserts directly into the target cell membrane and thereby should play a key role during the fusion between the viral and the cellular membrane.

Mutations in the NH_2-terminal fusion domain of HIV gp41 and SIV gp32, replacing hydrophobic residues with charged residues, completely inhibited the fusion process without affecting gp160 processing, receptor binding, and fusion protein expression (Kowalski et al., 1987; Freed and Risser, 1990; Bosch et al., 1989). Studies by Kowalski et al. (1987) and Freed and Risser, (1990) show that the fusogenic capacity of the viral glycoprotein is not limited to the presence of a hydrophobic stretch at the N terminus of its transmembrane subunit. These observations suggest that, whereas the so-called fusion domain is important for the fusogenic properties, other domains might play a role as well. In HIV-1, for example, mutations in the third hypervariable region of the external glycoprotein, the V3 loop (Freed and Risser, 1990), alter syncytia formation without affecting the capacity of the gp160 to bind to its receptor. This observation is of crucial importance, since several neutralizing antibodies against V3 loop, which is the major immunogenic epitope of HIV-1, inhibit viral penetration but does not affect attachment to its receptor. This "postbinding" neutralization is thought to act at the level of the fusion step (Skinner et al., 1988; Looney et al., 1989).

A possible way to elucidate the molecular mechanism of membrane fusion is to synthesize peptides corresponding to the amino terminus of viral fusion protein and to study their interaction with model membranes. Synthetic peptides corresponding to the fusion domain of influenza have a high tendency to interact with phospholipid membrane, promote fusion of small, unilamellar vesicles, and induce leakage of liposome-entrapped solutes (Wharton et al., 1988; Murata et al., 1987). The peptide adopts an α-helical conformation when bound to vesicles (Lear and DeGrado, 1987). Similarly, peptides representing the NH_2-terminal extremity of HIV-1 (Rafalski et al., 1990) or SIV (Martin et al., 1991) adopt α-helical structures in the presence of vesicles and induce leakage of liposome internal content.

Membrane fusion requires the destabilization of the lipid bilayer. Nonbilayer structures, responsible for membrane fusion, have been described (Verkleij, 1984), but the mechanism by which peptides or proteins could generate such structures and induce fusion remains unknown. In fact, there is no obvious relationship between the conformational properties of the fusion domain and the destabilizing events that occur prior to fusion.

Interaction of viral fusion peptides with the host–cell membrane was studied recently with computer analysis (Brasseur et al., 1990). The amino acid sequence of the viral envelope glycoproteins was analyzed by the Eisenberg et al. (1982) procedure. The region surrounding the cleavage site consists of a highly hydrophilic domain immediately followed by a hydrophobic region, the so-called fusion peptide. The mode of insertion of the fusion peptide in the lipid monolayer was determined using a theoretical analysis procedure, allowing the assembly of membrane components (Brasseur et al., 1990), and led to the conclusion that the fusogenic helices were oriented obliquely with respect to the lipid–water interface. This rather unusual orientation could be envisioned as a prerequisite to membrane destabilization and fusogenic activity of the peptide. The validity of these theoretical assumptions about the role of the orientation of the fusion peptide in the fusion mechanism has been confirmed by site-directed mutagenesis of the SIV_{mac} gp32 fusion domain and by expression in mammalian cells using vaccinia vector (Horth et al., 1991). Similar conclusions were reached with BLV (Vonèche et al., in press). Here we

discuss experimental evidence that the structure and the orientation of the NH_2-terminal SIV_{mac} gp32 peptide into the lipid bilayer plays an essential role in the peptide fusogenic activity.

Liposomes are stable structures that do not fuse spontaneously. Fusion assays are available and synthetic peptides corresponding to the fusogenic domain of viral envelope glycoproteins show a fusogenic activity even when not integrated in the whole glycoprotein structure (Martin et al., 1991; Rafalski et al., 1990; Wharton et al., 1988). We report here about the interactions between synthetic peptides corresponding to the fusogenic sequence of the SIV_{mac} gp32 and model membranes. Lipid mixing and lipid destabilization were measured using available assays based on fluorescence resonance energy transfer (Struck et al., 1981). Polarized infrared spectroscopy spectra gave information about the secondary structure and the mean orientation of peptides in the lipid bilayer (Goormaghtigh et al., 1990).

LIPID MIXING ASSAYS

The lipid mixing between unilamellar vesicles induced by the SIVWT peptides (Fig. 21–1) was studied as a function of temperature, pH, liposome composition, and size using fluorescence energy transfer between NBD-PE and Rh-PE (Struck et al., 1981). Addition of the wild-type SIV peptides to small unilamellar vesicles (SUV) of dioleoylphosphatidylcholine (DOPC) or phosphatidylcholine (PC)/phosphatidylethanolamine (PE)/sphingomyelin (SM)/cholesterol (chol) (26.3%, 26.3%/26.3%/21% w/w) induces a rapid lipid mixing between labeled and unlabeled vesicles (Fig. 21–2). When DOPC is replaced by palmitoyloleoyl phosphatidylcholine (POPC), no fusion is observed (Table 21–1). The presence of cholesterol in DOPC vesicles (molar ratio DOPC: Chol of 1:.5 or 1:.75) reduces the fusion activity of the SIV peptides (Table 21–1). Lipid mixing is maximum for SUV made of PC/PE/SM/Chol, the major neutral lipids of a typical mammalian plasma cell. Surprisingly, the extent of fusion decreases when the peptide length increases. For all the liposome compositions tested, the fusion is more efficient at 37°C than at 20°C (Table 21–2). SIV, as well as HIV, is known as a pH-independent virus (Stein et al., 1987). Those viruses fuse with the cell membrane at neutral pH but may fuse with endosome as well (Marsh and Helenius, 1989). We have tested the fusogenic activity of the SIVWT peptide at different pHs. The ability of SIV peptides to induce lipid mixing is comparable at pH 7.4 and pH 4.0 (data not shown).

Fusion at 37°C was also observed with the large unilamellar vesicles (LUV) prepared by extrusion (Hope et al., 1985), composed of PC/PE/SM/Chol (26.3%/26.3%/26.3%/21% w/w) or PC/PE (50%/50% w/w) (Fig. 21–3); their curvature and stability is expected to better mimic the plasma membrane bilayer. When PE is replaced with PC (PC/SM/Chol: 52.6%/26.3%/21% w/w) the percentage of lipid mixing is reduced from 40% to 15%. No lipid mixing was observed with DOPC LUV. For each lipid composition, the lipid mixing maximum is reached after less than 5 minutes, indicating that the fusion is a rapid process. The presence of PE in the lipid bilayer seems to enhance the process at 37°C and pH 7.4. PE is known to form hexagonal phases

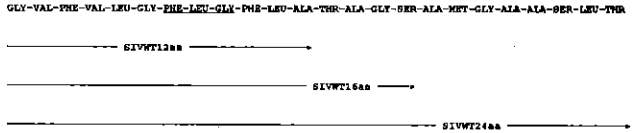

Fig. 21–1. Amino acid sequences of peptides from the gp32 N terminus of SIV_{mac} (BK28).

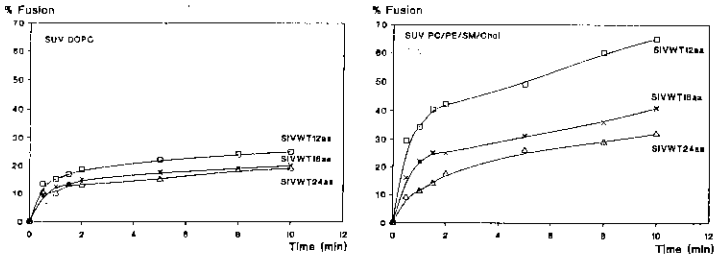

Fig. 21–2. Time course of small unilamellar vesicles (SUV) fusion induced by SIVWT peptides. A 1/9 mixture of labeled and unlabeled vesicles was incubated at pH = 7.4 and 37°C (total lipid concentration, 3×10^{-4} M); no change in fluorescence was observed with time. At time zero, 1.3×10^{-5} M of the peptide in DMSO was added under stirring, and the change in fluorescence at 530 nm was monitored. Dimethylsulfoxide (DMSO) up to 2% (v/v), the maximal concentration introduced in the system, does not affect the fluorescence. The experimental conditions are the same for the different lipid compositions DOPC (left) and PC/PE/SM/Chol (26.3%/26.3%/26.3%/21% w/w) (right) liposomes.

TABLE 21–1. Percentage of Fusion after 10 Minutes[a]

Liposomes SUV	DOPC (%)	DOPC/Chol 1/0.25 (%)	DOPC/Chol 1/0.50 (%)	DOPC/Chol 1/0.75 (%)	POPC (%)
SIVWT12aa	20	18	4.5	7.5	0

[a]Fusion was obtained with small unilamellar vesicles of different lipid composition at 20°C and pH 7.4, after addition of SIVWT12aa peptide. The total lipid concentration is 3×10^{-4} M, and the molar lipid:peptide ratio is 25.

TABLE 21–2. Percentage of Small Unilamellar Vesicles (SUV) Fusion after 10 Minutes[a]

Liposomes	SIVWT12aa (%)	SIVWT16aa (%)	SIVWT24aa (%)
SUV DOPC			
20°C	20	20	20
37°C	25	25	25
SUV PC/PE/SM/Chol			
20°C	20	20	15
37°C	65	41	32

[a]Fusion was induced by SIVWT peptides at 20°C and 37°C. The total lipid concentration is 3×10^{-4} M, and the molar lipid:peptide ratio is 25.

that are transient lipidic structures formed during the fusion mechanism (Ellens et al., 1989).

AQUEOUS CONTENTS MIXING AND LEAKAGE OF AQUEOUS CONTENTS

Ideally liposome fusion should be monitored by the mixing of both lipid and aqueous contents. To study the mixing and/or leakage of aqueous contents of the liposomes, we used the ANTS-DPX assay (8-aminonaphthalene-1,3,6-trisulfonic acid sodium salt [ANTS]-p-xylylenebis[pyridinium] bromide [DPX]) developed by Ellens et al. (1984). Briefly, for leakage experiments, fluorophore (ANTS) and quencher (DPX) are coencapsulated in the vesicles, and fluorescence increase corresponds to a release of vesicle contents into the external medium. For aqueous contents mixing, liposomes containing ANTS are mixed with liposomes containing DPX. Mixing of internal contents during vesicle fusion causes a decrease in fluorescence intensity. The mixing of aqueous contents cannot be observed satisfactorily if a rapid leakage of the vesicle content occurs.

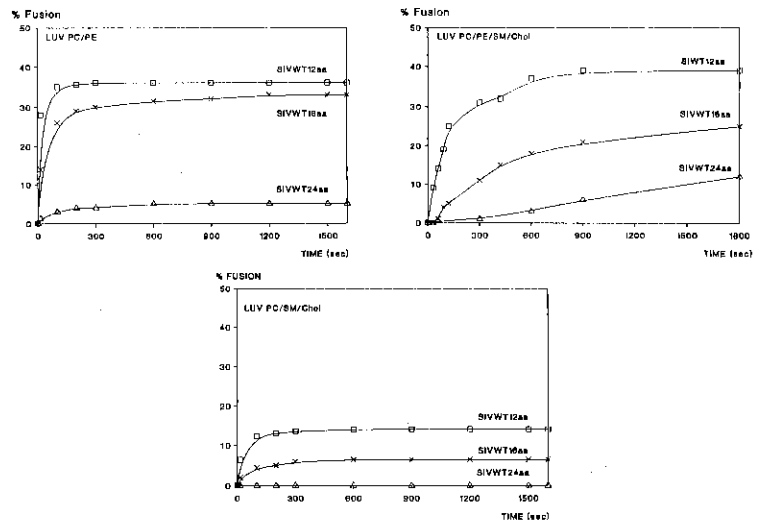

Fig. 21–3. Fusion of large unilamellar vesicles (LUV) induced by the SIVWT peptides at various lipid compositions. Labeled and unlabeled vesicles were mixed at a molar ratio of 1/9. At time 0, the peptide in DMSO was added and the increase in fluorescence, because of a decrease in resonance energy transfer following liposome–liposome fusion, was monitored at 530 nm, pH 7.4, and 37°C. DMSO up to 2% (v/v), the maximal concentration introduced into the system, does not affect the fluorescence. The experimental conditions are the same for all the lipid compositions tested. The total lipid concentration was 3×10^{-4} M and the peptide concentration 1.3×10^{-5} M. The molar lipid/peptide ratio is 25.

Addition of the SIVWT peptides to LUV PC/PE/SM/Chol containing coencapsulated ANTS and DPX induces a rapid increase of the fluorescence, demonstrating the fast leakage of internal contents, and explains why we were unable to follow the mixing of the liposomes aqueous contents (Fig. 21–4). SIV peptides cause a rapid release of ANTS-DPX from liposomes, possibly as a consequence of their destabilizing effect on the lipid organization. The peptide-induced leakage depends on the lipid composition of the vesicles. It was extensive with liposomes containing PE in their bilayer (PC/PE/SM/Chol or PC/PE [1/1]), whereas in the absence of PE (LUV PC/SM/Chol or DOPC) only a small percentage of leakage was observed (±10%). Typical leakage curves are presented in Figure 21–4. An initial burst of leakage is followed by a region of slow change after which the extent of leakage appears to reach a plateau. Comparison of lipid mixing and leakage assays demonstrates that the two processes are pH independent and that the kinetics are qualitatively similar and simultaneous. As for the lipid mixing assay, the ability of peptide to promote leakage of LUV is dependent on the peptide length: shorter peptide induces more extensive leakage than longer peptide.

The observed leakage of small solutes like

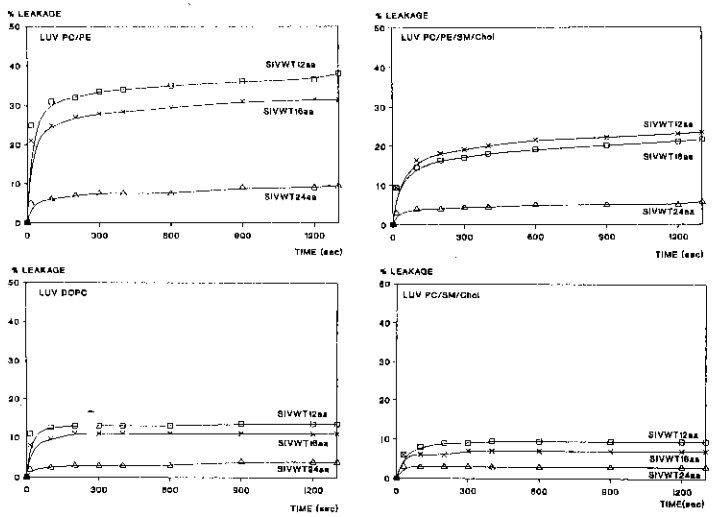

Fig. 21–4. SIVWT peptide-induced leakage of aqueous contents of large unilamellar vesicles (LUV) monitored with the ANTS/DPX assay at neutral pH and 37°C; 13 μM peptide was added at t = 0 in 3×10^{-4} M ANTS/DPX liposome suspension. The increased fluorescence caused by the dilution of ANTS in the suspension was monitored at 520 nm.

ANTS or DPX could be due to the formation of hexagonal phase via inverted micellar intermediates during the lipid bilayer fusion. The formation of those transient lipid structures could allow the passage of small molecule through the interlamellar attachment sites.

STRUCTURE AND ORIENTATION OF THE FUSOGENIC PEPTIDES IN THE LIPID BILAYER

Attenuated total reflection (ATR) Fourier-transform infrared spectroscopy of thin hydrated films of protein or peptide inserted into a phospholipid bilayer has been shown to provide useful information about the secondary structure of the protein. The analysis of the amide I band of deuterated samples by Fourier self-deconvolution (Fringeli and Günthard, 1981) followed by a curve fitting provides a correct estimation of the secondary structure content with a standard deviation of 8.6% when x-ray structures are taken as reference (Goormaghtigh et al., 1990).

ATR spectroscopy offers several advantages: It requires small amounts of material (typically 10 μg), it does not require knowledge of the protein concentration, it is not disturbed by highly turbid samples, such as large membrane fragments or precipitates, and it allows the orientation of the secondary structures with respect to the lipid bilayer plane to be determined (Goormaghtigh et al., 1990).

Infrared spectra of SIVWT peptides alone obtained by direct evaporation of a concentrated solution in dimethyl sulfoxide (DMSO)

TABLE 21–3. Proportion of the Different Secondary Structures of SIVWT12aa, SIVWT16aa, and SIVWT24aa

Sample	α-Helix (%)	β-Sheet (%)	Random (%)
SIVWT12aa	0	67	33
+ DOPC	48	34	19
SIVWT16aa	9	76	15
+ DOPC	39	58	3
SIVWT24aa	9	53	38
+ DOPC	45	20	35

[a]Determined in the absence and in the presence of lipid (SUV of DOPC). The molar lipid:peptide ratio is 65.

on the ATR plate revealed that the peptides adopt a β-sheet conformation (Table 21–3). SIVWT peptides dissolved in DMSO solvent and, injected in water, keep this β-sheet conformation (Fig. 21–5). Isolation of fused DOPC SUV on a sucrose gradient (30%–2%) revealed that this minor population displays an α-helical structure (Fig. 21–5) characterized by the appearance of a new large peak centered around 1,650 cm^{-1}. This significant increase in the α-helix structure (40% to 50%) content is accompanied by a decrease of the β-sheet content (Table 21–3). The percentage of α-helix content is proportional to the lipid:peptide ratio (Fig. 21–6, Table 21–4). It is not known whether this percentage reflects the proportion of each peptide in an α-helical conformation or the proportion of peptides with 100% α-helix structure.

From the spectra recorded with the incident light polarized at 90° and 0°, the dichroic ratio $R_{atr} = A_{90}/A_0 = 1.14$, corresponding to the helical structure of the 12 amino acid long peptide, indicates that the α-helix is neither parallel nor perpendicular to the lipid acyl chain but adopts an intermediate orientation in the lipid bilayer (Martin et al., 1991). The dichroic ratio $R_{atr} = 2.3$ associated to the β-sheet structure corresponds to an orientation parallel to the lipid bilayer. These orientations did not change whatever the lipid:peptide ratio, suggesting that two separate peptide populations exist: one penetrating into the lipid bilayer in an α-helical structure and the other remaining in the aqueous phase as a β-sheet.

DISCUSSION

Studies on the interaction of natural and synthetic peptides with membranes, using liposomes as model systems, have contributed to establish the involvement of a specific domain of the viral envelope glycoprotein in the virus–cell fusion. This domain is located at the amino-terminal extremity of the transmembrane glycoprotein and referred to as the fusion peptide.

Our results show that synthetic peptides of 12, 16, and 24 residues corresponding to the N-terminal domain of the transmembrane glycoprotein (gp32) of SIV are able to promote fusion of lipid bilayers, demonstrating that at least a part of the fusogenic properties attributed to gp32 depend on a limited stretch of its N-terminal domain. Lipid mixing is paralleled by changes in membrane permeability: small solutes rapidly leak out of the vesicles. A similar observation was made with influenza peptides (Wharton et al., 1988) and more recently with HIV peptides (Rafalski et al., 1990), giving further experimental support for the role of the N-terminal fusogenic sequence in viral fusion. The loss of vesicle contents during fusion could be the result of the H_{II} phase formation or a local change in lipidic organization caused by the peptide.

In contrast to studies on influenza, where longer peptides were more fusogenic than shorter one, 12 and 16 residue peptides are highly fusogenic, the 12 amino acids being the most efficient. This result suggests that in the case of SIV (and perhaps other viruses) the so-called fusion domain could be shorter. A theoretical model has been proposed to explain the higher fusogenic capacity of the 12 residue peptide (Brasseur et al., 1990). When relative positions and orientations of the peptides at the lipid–water interface were calculated for increasing peptide length, a maximum deviation between the orientation of the helix axis and the lipid–water interface occurs for the 12 residue-long peptide; the deviation is minimum for the 24 residues peptide (Brasseur et al., 1990). It should be kept in mind

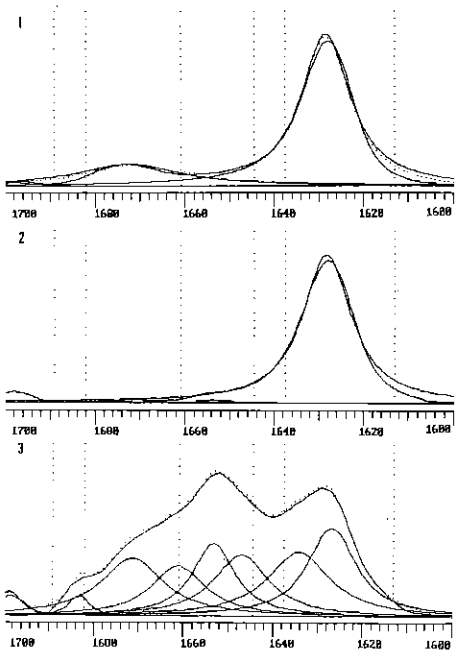

Fig. 21–5. Curve fitting of the amide I′ region of the SIVWT12aa at pH 7.4 in DMSO (**1**), in water (**2**), and in the presence of SUV DOPC (**3**). The result of the fitting appears under the curve. The vertical dotted lines define the region of the spectrum assigned to the different secondary structures. The frequency limits used were empirically determined (Cabiaux et al., 1989) and were as follows: 1662–1645 cm-1, α-helix; 1689–1682 cm-1 and 1637–1613 cm-1, β-sheet; 1644.5–1637 cm-1, random; 1682–1662.5 cm-1, β-turns. The sum of the components is represented by the dotted spectrum.

that when peptide is added to the liposome suspension, peptide–peptide interaction could be favored over peptide–lipid interactions. It is not clear how such interactions contribute to our results, and further experimental data are required to separate the contribution of peptide self-association and binding of peptide to the bilayer. Those peptide–peptide interactions could be stronger for the longer SIV_{mac} peptide and thus responsible for its weaker fusion activity.

The susceptibility of SUV toward fusion or destabilization is attributed to the strong curvature of their surrounding lipid bilayer. Here we show that LUV, depending on their lipid composition, are able to undergo fusion too. The decreased stability of LUV is probably due to the presence of PE. Indeed, unsaturated PE, a natural component of cell membranes, is known to undergo a phase transition from bilayer to hexagonal H_{II} phase, a structure that is thought to play a role in several membrane fusion processes (Ellens et al., 1989). The need for intrinsic destabiliza-

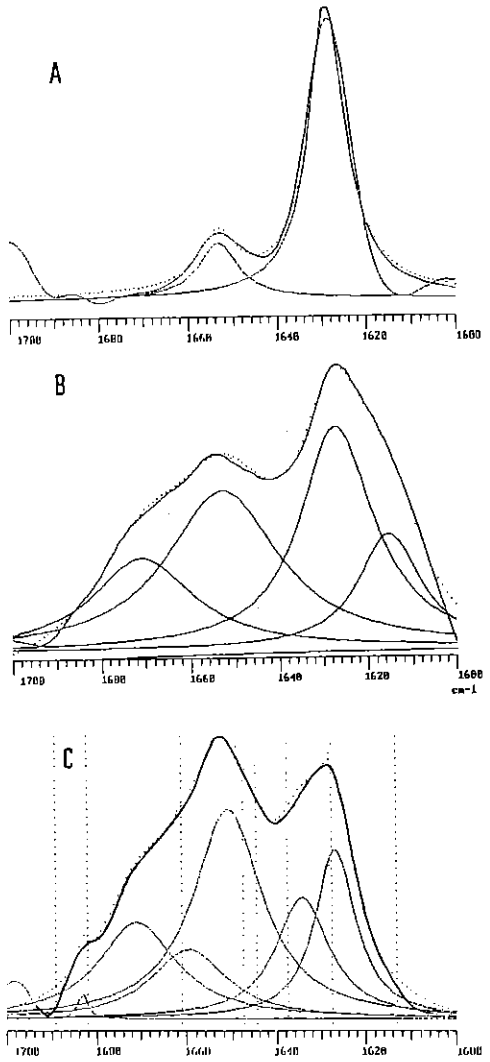

Fig. 21–6. Curve fitting of the amide I′ region of the SIVWT12aa at pH 7.4 in the presence of SUV DOPC at different lipid:peptide molar ratios (*r*). **A:** r = 18; **B:** r = 36, **C:** r = 65. The results of the fitting appear under the curve.

TABLE 21–4. Proportion of the Different Secondary Structures of SIVWT12aa in the Presence of Lipid (SUV of DOPC) for Different Lipid:Peptide Ratios

SIWT12aa + DOPC lipid:peptide ratio	α-Helix (%)	β-Sheet (%)	Random (%)
18	17	83	0
36	35	47	18
65	48	34	19

tion factors (PE or surface curvature) in vesicle fusion suggests a possible role for those additional factors in viral membrane fusion. Comparable observations are made with other virus such as Sindbis virus or vesicular stomatitis virus (Yamada and Ohnishi, 1986; Scheule, 1987).

Infrared spectroscopy data indicate that fusogenic peptides undergo a conformational transition from β-sheet to α-helix when interaction with lipids occurs. A similar β-sheet to α-helical transition was demonstrated by circular dichroism with synthetic peptides corresponding to the NH_2 terminus of influenza HA_2 (Lear and DeGrado, 1987). This transition does not seem to be a specific property of the fusion domain, since it has also been observed with a 30 amino acid peptide designed to mimic the properties of viral fusion protein (Subbarao et al., 1987) or in signal sequence peptides (Roise et al., 1986; Goormaghtigh et al., 1989).

As observed in the spectra, even at a high lipid:peptide ratio, there are two major well-separated spectral components in the α-helix and β-sheet spectral domains, showing that the conversion to α-helix is uncomplete. The occurrence of two spectral components should reflect the presence of two separate peptide populations, one penetrating into the lipid bilayer in an α-helical structure and the other remaining in the aqueous phase as a β-sheet. Indeed, it is hard to believe that two well-defined α-helix and β-sheet conformations could coexist within the same short sequence, and we see no obvious reason why the peptide should gradually enlarge its α-helix domain while the lipid:peptide ratio is increased.

The hydrophobicity increases along the helix from the N terminus to the C terminus of the SIV peptide. This asymmetrical distribution of hydrophobicity has been demonstrated to be associated with the fusion domain of a series of viruses (Brasseur et al., 1990) and is generally not observed in transmembane segments and surface-seeking helices of proteins. According to our previous computer analysis studies, this asymmetrical distribution of residues might induce an unusual orientation of the fusogenic peptide at the lipid–water interface. A tilt angle of 52° from the normal to the lipid bilayer was calculated for SIV (Brasseur et al., 1990). This is in accordance with the mean angle of orientation found by infrared spectroscopy in this work.

However, even if it is tempting to conclude that fusogenic capacity depends on the orientation of a peptide in the lipid bilayer, it should be kept in mind that infrared spectroscopy is unable to discriminate between a fixed uniaxial orientation and a mixture of two (or more) populations with different orientations giving an average oblique orientation. The prediction approach gives only a static view of the phenomenon, and the small differences in calculated energies for various orientations could reflect the fact that the fusogenic segment is actually changing its orientation quickly around a mean orientation. We recently synthesized several peptides having the same length and the same hydrophobicity as the gp32 NH_2-terminal domain but for which the calculated orientation into the lipid bilayer has been modified. A good correlation was observed between the oblique orientation of the peptides and their capacity to induce liposome fusion (to be published). Another way to verify this correlation is to integrate these mutations in the SIV gp160 by site-directed mutagenesis via a vaccinia virus vector. The fusogenic activity of the wild-type and the mutant glycoproteins were tested after infection of T4 lymphocytic cell lines by the recombinant vaccinia virus. Mutations that modified the oblique orientation did not alter the fusogenic properties. The results support the hypothesis that oblique orientation is important for fusogenic activity (Horth et al., 1992).

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Reconstitution of Human Immunodeficiency Virus Envelope

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INTRODUCTION

Cell surface receptor recognition and subsequent events (fusion, uncoating) leading to delivery of the viral genome into the cell cytoplasm are the first crucial steps in the cell infection process. Site-directed mutagenesis as well as the comparison of naturally occurring variants have demonstrated the importance of several domains of the envelope glycoproteins in the virus–host cell fusion process (Boggs et al., 1989; Bosch et al., 1989; Freed et al., 1990; Gething et al., 1986; Helseth et al., 1990; Kowalski et al., 1987; Olshevsky et al., 1990). In a large number of cases, the cleavage of a glycoprotein precursor generates an N-terminal hydrophobic domain (the so-called fusion domain) essential for the fusogenic activity (Bosch and Pawlita, 1990; Freed et al., 1989; Le, et al., 1988; McCune et al., 1988).

Reconstitution of the viral envelope to form virosomes has been used as a convenient tool to elucidate the largely unknown mechanism of virus–host cell membrane fusion (Citovsky and Loyter, 1985; Eidelman et al., 1984; Gould-Fogerite et al., 1988; Marsh et al., 1983; Metsikkó et al., 1986; Scheule, 1986; Stegmann et al., 1987). However, the fusogenic properties depend on the reconstitution procedure used, which means that this strategy is not as straightforward as originally thought. As a general protocol seems not available, structural and functional similarities between virosomes and the original viral envelope have to be carefully examined in each case.

Another important potential of virosomes is their use as subunit vaccine carriers (Ada, 1989; Gregoriadis, 1990; Morein et al., 1978; Morein and Simons, 1985; Patterson and Oxford, 1986). It is highly recommended that vaccines against retroviruses such as HIV or HTLV-I, do not contain any potentially hazardous genetic material but do contain the minimal epitopes necessary for protection. With the advent of genetic engineering of viral proteins, this goal seems now at hand. However, subunit vaccines suffer from serious limitations, mainly a poor immunogenicity in the absence of adjuvants (Morein and Simons, 1985). The only adjuvant licensed for human beings is alum, which is not very efficient. New formulation systems have already been used in animals, which include liposomes (Gregoriadis, 1990; Morein and Simons, 1985). Liposomes could have the advantage of being well tolerated (Gregoriadis, 1988; Lopez-Berestein and Fidler, 1989), because high amounts of phospholipids have been used for lipophilic drug deliv-