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I Martin, H Schaal, A Scheid and J M Ruysschaert

Lipid Membrane Fusion Induced by the Human Immunodeficiency Virus Type 1 gp41 N-Terminal Extremity Is Determined By Its Orientation in the Lipid Bilayer

ISABELLE MARTIN,†₁ HEINER SCHAAL,² ANDREAS SCHEID,² AND JEAN-MARIE RUYSSECHAERT₁

Laboratoire de Chimie-Physique des Macromolécules aux Interfaces, Université Libre de Bruxelles, 1050 Brussels, Belgium, and Biologisch-Medizinisches Forschungszentrum und Institut für Medizinische Mikrobiologie und Virologie, Heinrich-Heine-Universität Düsseldorf, D-40225 Düsseldorf, Germany²

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The amino-terminal extremity of the human immunodeficiency virus type 1 transmembrane protein (gp41) is thought to play a pivotal role in the fusion of virus membranes with the plasma membrane of the target cell and in syncytium formation. Peptides with sequences taken from the human immunodeficiency virus type 1 gp41 fusogenic (synthetic peptides SPwt and SP-2) and nonfusogenic (SP-3 and SP-4) glycoproteins adopt mainly a β-sheet conformation in the absence of lipid, as determined by attenuated total reflection Fourier transform infrared spectroscopy, and after interaction with large unilamellar liposomes, the β-sheet is partly converted into an α-helical conformation. Peptides SPwt and SP-2 but not SP-3 or SP-4 were able to promote lipid mixing as assessed by fluorescence energy transfer assay and dye leakage in a vesicle leakage assay. By using polarized attenuated total reflection Fourier transform infrared spectroscopy, SPwt and SP-2 were found to adopt an oblique orientation in the lipid membrane whereas SP-3 and SP-4 were oriented nearly parallel to the plane of the membrane. These findings confirm the correlation between the membrane orientation of the α-helix and the lipid mixing ability in vitro. Interestingly, the data provide a direct correlation with the fusogenic activity of the parent glycoproteins in vivo.

The human immunodeficiency virus type 1 (HIV-1) envelope glycoprotein precursor gp160 is proteolytically cleaved by a cellular enzyme into a surface glycoprotein (gp120) and a transmembrane glycoprotein (gp41). This cleavage generates in gp41 the exposure of an N-terminal highly hydrophobic amino acid stretch, which has been proposed to interact with the lipid membrane during the fusion event. Involvement of the N terminus of the glycoproteins of different enveloped viruses has been confirmed by mutational analysis: mutations which disrupt the distribution of the hydrophobic amino acids in the N terminus of the influenza virus hemagglutinin (HA) (8), gp41 of HIV-1 (5, 16, 29), and gp32 of simian immunodeficiency virus (SIV) (1) inhibit syncytium formation in transfected cells. The mode of interaction of the fusion peptide with the lipid membrane, in particular the mechanism of insertion into the membrane and the mechanism by which this facilitates the fusion between membranes, is not fully understood. The available evidence suggests that peptide hydrophobicity is important but that other factors such as the distribution of the amino acids on a helical motif are also involved, as well as the orientation of the peptide at the lipid/water interface (7, 14, 22, 35).

A possible way to elucidate the molecular mechanism of membrane fusion is to study the interaction of synthetic peptides corresponding to the amino terminus of viral fusion proteins with model membranes. Synthetic peptides corresponding to the N-terminal segment of the influenza virus HA₃ (18, 24, 27, 34) have been shown to interact with the lipid bilayer and to promote fusion of lipid vesicles. Similar observations have been made with SIV and HIV-1 fusion peptides (20, 25, 26).

In an attempt to establish a relationship between peptide fusion activity, structure, and orientation, we have recently investigated in a liposomal system the activity of a 12-residue synthetic peptide corresponding to the N-terminal extremity of SIV gp32 and modified SIV fusion peptides and have found that the oblique membrane insertion of the SIV fusion peptide is an essential requirement for membrane fusion (22). It is likely that this oblique orientation alters the parallelism between the phospholipid acyl chains, giving rise to new lipid phases which we now think to be associated with the initial events of membrane fusion.

Using an expression vector for the HIV-1 glycoprotein, Schaal et al. (29) have recently identified several deletions in the HIV-1 gp41 fusion peptide which abolish syncytium formation of transfected cells. In the present study, we asked whether the same mutations, when introduced into synthetic peptides, lead to corresponding changes in their physical interactions with membrane lipids and whether we can validate the correlation between peptide orientation and fusogenic activity. To correlate the activity of synthetic peptides with the activity of mutant glycoprotein, three mutant peptides corresponding in sequence to the N-terminal extremity of one active glycoprotein lacking two amino acids (SP-2) and two inactive glycoproteins lacking three and four amino acids (SP-3 and SP-4) were compared with a peptide with the wild-type sequence (SPwt). The membrane activity of the peptides in vitro conforms with the fusion activity of the parent glycoprotein. The presence or absence of fusion activity is correlated with the orientation of the peptides in the lipid bilayer established by Fourier transform infrared spectroscopy (FTIR).

MATERIALS AND METHODS

Materials. Egg phosphatidylethanolamine (PE) and egg phosphatidylcholine (PC) were purchased from Sigma Chemical Co., St. Louis, Mo. N-(Nitrobenzoyl-2-oxa1,3-diazol)phosphatidylethanolamine (NBD-PE) and N-(lissamine rhoda-
amine B sulfonylphosphatidylethanolamine (Rh-PE) were from Avanti Polar Lipids Inc., Birmingham, Ala. 8-Amino-9-naphthol-1,2,3-trisulfonic acid sodium salt and p-xylenebis(phenyldrazine) (pridinium) bromide were from Molecular Probes Inc., Junction City, Oreg.

Calcine (Sigma) was purified by column chromatography on Sephadex LH-20 (Pharmacia). Calcine was loaded on the column as a sodium salt solution and eluted with water at neutral pH. The concentration of calcine was determined spectrophoto metrically by using 7.0 × 10⁻⁵ M as the molar extinction coefficient at 492 nm (12).

High-pressure liquid chromatography-purified synthetic peptides in their amide form were purchased from Quality Controlled Biochemicals, Inc., Hop kinton, Mass.

**Vesicle preparation.** Multimellar vesicles (MLV) were obtained by vortexing a 1 mg/ml NaCl solution (10 mM Tris, 0.1 mM EDTA, 0.1 mM NaCl, pH 7.4). Large unimellar vesicles (LUV) were prepared by the extrusion procedure of Hope et al. (13) with an extruder (Lipex Biomembranes Inc., Vancouver, Canada). Briefly, frozen and thawed MLV were extruded 10 times through two stacked polycarbonate membranes with a pore size of 0.1 μm (Nuclepore Corp., Pleasanton, Calif.).

**Lipid mixing.** Lipid mixing was determined by measuring the fluorescence intensity change resulting from the fluorescence energy transfer between two probes, NBD-PE and Rh-PE, inserted into the lipid bilayer, as described by Struck et al. (30). Fluorescence was monitored with a model 8000 spectrofluorimeter (SLM Instruments Inc., Urbana, Ill.) with excitation and emission slits of 4 nm. Probes were added to the lipid film, and LUV were prepared as described above. Spectra were recorded both before and after (at a probe ratio) each spectrum was taken as 0% fluorescence, and the 100% fluorescence value was determined with respect to the background spectrum of vesicles prepared with 0.06% of each fluorescent phospholipid. The suspensions were excited at 470 nm, and the NBD fluorescence was recorded at 530 nm. The addition of Triton X-100 to a final concentration of 0.1% (vol/vol) above LUV containing both probes at 0.6% (mol/mol) each were mixed in an acrylamide gel poured on a nitrocellulose membrane. The gels were then incubated with the lipid mixtures run for 3 h at 100°C and dried at room temperature. The nitrocellulose membranes were exposed to X-ray film (Kodak X-Omat5) with an exposure time of 2 days. The intensity of the bands corresponding to the lipid mixtures was determined by densitometry.

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**RESULTS**

**Peptide-induced lipid mixing and dye leakage.** The 16-amino-acid synthetic peptide (SPw7) corresponding to the fusogenic sequence of the HIV-1 gp41 has been shown to promote the lipid mixing of PE-containing LUV (PE/PC molar ratio = 1:1) (19) as determined by resonance energy transfer (30).

Here, we compared three peptides corresponding to deletion mutants of the HIV-1 gp41 N-terminal extremity with SPw7 (Fig. 1). Like SPw7, SPw2 promoted the fusion of LUV at a lipid/peptide molar ratio of 25, neutral pH, and 37°C. In fusion was observed in the presence of SPw7 (Fig. 1). Like SPw7, SPw2 promoted the fusion of LUV at a lipid/peptide molar ratio of 25, neutral pH, and 37°C. No fusion occurred with SPw3 and SPw4 under the same experimental conditions (Fig. 2A). This loss of fusion activity with deletions at the N terminus is not likely to be due to the decrease in the overall lengths of the inactive 12- and 13-amino-acid peptides, because a 13-amino-acid peptide resulting from the C-terminal deletion of the 16-amino-acid HIV fusion peptide showed a high fusion activity, as did a 12-amino-acid peptide of the analogous SIV fusion peptide (20) (Fig. 2A).

It has been shown in vivo that the cotransfection of a nonfusogenic mutant protein with the fusogenic wild-type gp41 results in a reduced cell-fusing activity of HeLa-T4+ cells (29). We have therefore analyzed the capacity of the nonfusogenic peptides SPw3 and SPw4 to interfere with the activity of
the fusogenic peptides SPwt and SP-2. Addition to the vesicles of SPwt premixed with SP-3 or SP-4 at a 1:1 molar ratio resulted in the same effects as produced by SPwt alone, and preaddition of SP-3 or SP-4 to the vesicles did not affect membrane mixing induced by SPwt or SP-2, indicating that the nonfusogenic peptides were unable to interfere with the activity of fusogenic peptides under these conditions in vitro (data not shown).

The interaction of peptides with phospholipid vesicles was further investigated by measuring the ability of the peptides to induce leakage of calcine from PC/PE vesicles. The relative efficiency of these peptides in inducing leakage correlates with their fusogenic activity; the fusogenic peptides SPwt (16 and 13 amino acids) and SP-2 exhibited a high efficiency in inducing dye leakage, while the nonfusogenic peptides SP-3 and SP-4 did not destabilize the LUV envelope (Fig. 2B).

Conformation and orientation of the modified peptides. The peptides were deposited on the geranium crystal from a DMSO solution (1 mg/ml). At pH 7.4 and in the absence of lipid, the HIV-1 peptides adopt a β-sheet structure characterized by a maximum at 1,628 cm⁻¹ (Fig. 3A). Estimation of the secondary structure by Fourier self-deconvolution and curve fitting confirmed this predominance of β-sheet structure (Table 1) for all the HIV-1 fusion peptides.

LUV of PC/PE (1:1 molar ratio) were incubated with the peptides for 1 h at 37°C, and the proteoliposomes were separated from the free peptides on a Sephadex G-50 column. In contrast to lipid-free peptide, proteoliposomes displayed FTIR spectra (Fig. 3B and C) with an intense band, centered at 1,735 cm⁻¹, corresponding to the COOH stretching vibration of the phospholipid ester bonds and a second band, centered at 1,652 cm⁻¹, corresponding to the amide I of peptides, indicating that the HIV-1 peptide preferentially adopted an α-helical conformation. There was a continuing shift to the α-helical conformation with the increase of the lipid-to-peptide ratio (Table 1).

The lipid-to-peptide ratio stated is the input ratio that was used to make the proteoliposomes before passage through the column, and the actual lipid-to-peptide ratio in the multilayer on the geranium plate has not been determined. However, the relative intensities of the peptide and lipid bands indicate a comparable degree of binding for the four peptides. This estimation was made by measuring the ratio Sₚ₋ₐₘᵢ₇₋ₐₐ₈₃ [Sₚ₋ₐₘᵢ₇₋ₐ₈₃ is the area of amide I measured between 1,680 and 1,600 cm⁻¹, and Sₚ₋ₐ₈₃₋ₐₐ₈₃ is the area of the lipid ν(C==O) band between 1,770 and 1,700 cm⁻¹] (9).

With the aid of the Fourier deconvolution and curve-fitting procedure, we found that the amide I band of HIV-1 fusion peptide is resolvable into three component bands with maxima near 1,665, 1,652, and 1,635 cm⁻¹ (Fig. 4). The component near 1,652 cm⁻¹ dominates the contours of the band (±85% of the total integrated intensity) and can be assigned to the amide I absorption of the α-helical domain of the peptide. The components near 1,665 and 1,635 cm⁻¹ together contribute some 15% of the integrated intensity of amide I band and are attributable to the amide I vibrations of the non-α-helical domain of the peptide (which are probably the COOH and NH₂ extremities). The three modified peptides present, within the limits of experimental accuracy, the same conformation in the absence and in the presence of lipids as the wild-type fusion peptide does (Table 1).

To determine the orientation of the secondary structures with respect to the lipid bilayer, spectra were recorded with orthogonally polarized incident light. A larger absorbance at 90° indicates a dipole oriented preferentially close to the perpendicular of the membrane plane, and, conversely, a larger absorbance at 0° indicates a dipole much closer to the parallel of the membrane plane. The orientational distributions of SPwt and SP-2 peptides are distinctly different from the orientation of SP-3 and SP-4 (Fig. 5). The difference spectra 90°−0° of SPwt and SP-2 reveal no deviation for the α-helix, suggesting that the α-helix is neither parallel nor perpendicular to the ATR element surface, as illustrated for the SP-2 peptide in Fig. 5A, while for SP-3 and SP-4, the 90°−0° difference spectra show a negative deviation for the α-helix, suggesting an orientation parallel to the lipid membrane, as shown for SP-3 in Fig. 5B.

As described in Materials and Methods, the curve fitting applied to the polarized spectra in the amide I region allows the evaluation of the dichroic ratio Rₐₐ₈₃ (Aₐ₈₃/Aₐ₉₅) for the α-helical structure and the calculation of the angle between the long axis of the α-helix and the germanium plate. For the helical structure associated with SPwt and SP-2, the calculated dichroic ratio of 1.3 ± 0.04 corresponds to an angle of 50 ± 5° between the α-helix and the germanium plate. The dichroic ratios associated with the SP-3 and SP-4 helical structures are 0.85 ± 0.03, corresponding to an angle of 0 ± 5° (Table 1). To
determine these orientations, a $27^\circ$ deviation angle between the $\alpha$-helix axis and the C=O transition dipole moment described by Rothschild et al. (28) was taken into account by introducing an order parameter $S_{\text{C=O}} = (3\cos^2 27^\circ - 1)/2$, so that $S_{\text{helix}} = S_{\text{measured}}/S_{\text{C=O}}$ (9, 11). The calculated angles between the helix axis and the bilayer are therefore minimum estimates, and an orientation of the helix axis closer to this perpendicular would result from considering other sources of disorder such as an imperfect parallelism between the bilayer and the germanium crystal surface.

Efforts have been made to characterize the orientation of the phospholipids in the bilayer in order to assess the overall membrane orientation on the germanium plate in the absence and the presence of peptide. In the absence of peptide, the peak of the phospholipid $\omega(CH_2)$ at 1,468 cm$^{-1}$ that appears on the dichroic ratio spectra (obtained by subtracting the $0^\circ$ spectrum from the $90^\circ$ spectrum) as a negative deviation demonstrates that the phospholipid acyl chains are oriented almost perpendicular to the germanium plate; i.e., the bilayer lies parallel to the germanium plate (data not shown). The dichroic ratio of the $\omega(CH_2)$ vibration at 1,468 cm$^{-1}$ is $0.80 \pm 0.05$ without or with peptide, and this is consistent with a well-ordered lipid film parallel to the plane of the germanium plate. The fact that peptide has no measurable effect on the lipid order is probably due to the low peptide-to-lipid molar ratio of 1:200 used in these experiments. Tamm and Tatulian (32) have demonstrated that only at peptide-to-lipid molar ratios up to 1:20 are small hydrophobic peptides able to disturb the orientational order of the lipid molecules to a significant degree.

**DISCUSSION**

The hydrophobic region located at the amino-terminal extremity of transmembrane envelope glycoproteins of several enveloped viruses (paramyxoviruses, orthomyxoviruses, and retroviruses) has been proposed to play a role in the fusion process and is referred to as the fusion peptide (for a review,
TABLE 1. Proportion of the different secondary structures of SPwt and mutant peptides in the absence and presence of LUV containing PC and PE at a 1:1 molar ratio

<table>
<thead>
<tr>
<th>Sample a</th>
<th>FTRIR secondary structure b</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>% α-helix (± 5%)</td>
</tr>
<tr>
<td>SPwt</td>
<td></td>
</tr>
<tr>
<td>in DMSO</td>
<td>5</td>
</tr>
<tr>
<td>r/L/P = 50</td>
<td>54</td>
</tr>
<tr>
<td>r/L/P = 100</td>
<td>85</td>
</tr>
<tr>
<td>r/L/P = 200</td>
<td></td>
</tr>
<tr>
<td>SP-2</td>
<td></td>
</tr>
<tr>
<td>in DMSO</td>
<td>7</td>
</tr>
<tr>
<td>r/L/P = 50</td>
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<tr>
<td>r/L/P = 100</td>
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<tr>
<td>r/L/P = 200</td>
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<td>SP-3</td>
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<td>in DMSO</td>
<td>7</td>
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<td>r/L/P = 50</td>
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<td>r/L/P = 100</td>
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<td>SP-4</td>
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<td>in DMSO</td>
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<td>r/L/P = 50</td>
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</table>

a r/L/P, lipid-to-peptide molar ratio before passage through a Sephadex G-50 column.

b The measurements were done after separation of the vesicle-bound peptide from free peptide on a Sephadex G-50 column.

c ND, not determined.

Expression vectors for mutant glycoproteins with deletions of 1 to 7 amino acids of the N terminus of gp41 have recently been constructed and tested for their ability to induce membrane fusion after transfection of HeLa-T4 cells by electroporation and microinjection. The efficiency of cell-fusing activity decreased drastically with deletion of 3 amino acids or more, suggesting that a specific N-terminal structure is required for fusogenic activity even though the deletion of up to 2 amino acids can be tolerated (29).

Since different studies on SIV gp32 have revealed a correlation between the orientation of the fusion peptide in the lipid membrane and its fusogenic activity (14, 22), we have now analyzed synthetic peptides corresponding in sequence to the gp41 N terminus of HIV-1 used by Schaal et al. (29) to ask whether the activity of these peptides and their interaction with lipid membranes correlates with the properties of the parent glycoproteins in vivo and with the membrane orientation of the peptides as predicted from our previous studies (14, 22).

The ability of the three mutant peptides to destabilize the lipid bilayer and to induce lipid mixing was tested and compared with the fusogenic activity of SPwt. SP-2 has an activity comparable to SPwt, while SP-3 and SP-4 have no capacity to destabilize the lipid bilayer or to induce membrane fusion. This loss of fusion activity cannot be ascribed to the shortening of the peptide per se, because Spwt13, a peptide equal in length to SP-3 but containing the N-terminal sequence of the 16-amino-acid Spwt, showed high fusion activity. These results with the model system are in good agreement with the in vivo data obtained by mutagenesis, which have also shown that the deletion of 2 amino acids has little effect on the fusogenic activity of gp41, while deletion of 3 and 4 amino acids drastically decreases the fusion activity in transfected HeLa-T4+ cells (29).

To better understand the insertion mode of these peptides in the lipid membrane, we have used ATR-FTIR to determine their secondary structure in proteolipid complexes. The three mutant peptides were found to have the same secondary structure in lipid bilayers as the wild-type peptide did. All peptides undergo a conformational transition to an α-helical structure when interacting with lipid. This β-sheet→α-helix transition has previously been demonstrated by circular dichroism for synthetic peptides corresponding to the N-terminal extremity of influenza virus (18) and by IR spectroscopy for the fusion peptides of HIV-1 (19, 26) and SIV (20, 21).

Because of the similarity of the secondary structure of wild-type and mutant peptides, this parameter clearly cannot account for the large functional differences found between the wild-type and mutant peptides in vitro and between their parent glycoproteins in vivo. Another important physical parameter which could potentially discriminate between functional and nonfunctional fusion peptides is the orientation in the membrane of the α-helices. A useful method for determining the orientation of α-helices of proteins or peptides in the lipid bilayer is ATR-IR with polarized light (9, 10, 33). Applying this method to the peptides, we found that deletion of 3 or 4 residues significantly changes the orientation of the α-helix relative to the lipid bilayer, lowering the angle relative to the plane of the membrane close to the parallel. This is in contrast to the oblique orientation of the wild-type peptide and SP-2. Thus, as subtle a change in sequence as the loss of 1 amino acid residue (from SP-2 to SP-3) can result in significant changes of the orientation of the helix, and this correlates with the change in the membrane fusion activity in vitro observed in this study and with the previously observed differences in fusion activity of the parent glycoproteins in vivo (29).

The results suggest that the oblique angle at which the hydrophobic fusion peptide is inserted into the lipid bilayer is an important parameter for its ability to destabilize the lipid organization and to promote membrane fusion. This further supports the correlation between membrane orientation and fusion that has previously been observed with SIV fusion.
peptides (22) and with mutations introduced into the fusion peptide of the entire SIV glycoprotein (14). A possible mechanism by which peptides inserted into membranes at an oblique angle may perturb bilayer packing is by increasing the negative curvature strain, thereby favoring the formation of inverted phases. There is some evidence that viral fusion peptides of SIV and influenza virus may promote fusion by this mechanism of phase destabilization (3, 4).

The good correlation observed between the in vitro and in vivo experiments confirms the complementarity and the potential of the two methods for examining the mechanisms by which peptide region of a transmembrane protein induce lipid bilayer fusion. Moreover, the similarity observed in the HIV and SIV systems (22) suggests the existence of a common fusion mechanism induced by the fusion peptides.

ACKNOWLEDGMENTS

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REFERENCES


FIG. 5. ATR-FTIR spectra of SP-2 inserted in LUV PC/PE (A) and SP-3 inserted in LUV PC/PE (B), recorded with 90° (bottom) and 0° (middle) polarization. The dichroism spectrum obtained by subtracting the 0° from the 90° recorded spectra is plotted at the top of the figure and expanded threefold in the ordinate direction. The bars indicate the protein amide I bands in the region of 1,600 to 1,700 cm⁻¹ and the phospholipid v(CH₂) at 1,467 cm⁻¹. The difference spectra are normalized to each other by setting to zero the net integral of the intensities of the ester C=O stretching bands of the Sn1 and Sn2 chains in the 1,710- to 1,760-cm⁻¹ region of the difference spectrum.
dent membrane fusion activity of a synthetic 20 amino-acid peptide with the same sequence as that of the hydrophobic segment of influenza virus hemagglutinin. J. Biochem. 102:957–962.
32. Tamm, L., and S. Tatulian. 1993. Orientation of functional and non func-
tional PTS permease signal sequences in lipid bilayer. A polarized attenu-