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Influence of the Mode of Insertion of SIV Peptides into Membranes on the Structure of Model Membrane as Studied by ^{31}P NMR

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The influence on model membrane organization of a fusion peptide of SIV and of a nonfusogenic mutant of this peptide was examined by molecular modeling and by ^{31}P NMR. The calculated mode of insertion of the fusion peptide shows that it adopts an oblique orientation towards the lipid–water interface and that this fusion peptide induces a destabilization of the bilayer structure of multilamellar vesicles as evidenced by ^{31}P NMR observations. The SIV mutant showing a more vertical insertion into lipid layers is unable to induce nonlamellar structures. This study reinforces the correlation between fusogenic activity, induction of structures not organized in extended bilayers, and calculated mode of insertion of peptides into lipid layers.

Most viral fusogenic protein contain a short N-terminal hydrophobic segment probably involved in the fusion event by interacting with the lipid membrane (1). Theoretical studies have predicted that the fusion peptide of SIV gp 32 would insert obliquely in the lipid bilayer (2). Functional and theoretical analysis of the SIV fusion peptide have demonstrated that the mode of insertion of the peptide into the membrane influences the fusogenic properties of the peptide. Mutations that modify the oblique orientation reduce the fusogenic activity (3). The fusion phenomenon certainly involves perturbations of the membrane organization. ^{31}P NMR is a suitable method for detecting structural modifications of phospholipids. We have examined with this technique the influence on model membrane of the SIV fusion peptide and of a modified non fusogenic peptide whose predicted orientation is more perpendicular to the lipid-water interface.

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Abbreviations: SIV, simian immunodeficiency virus; MLV, multilamellar large vesicles; DPPC, dipalmitoyl phosphatidyl choline; DPPE, dipalmitoyl phosphatidyl ethanolamine; DPPS, dipalmitoyl phosphatidyl serine; FID, free induction decay; FT, fourier transform.; Chol, cholesterol.

MATERIALS AND METHODS

Chemicals. The synthetic peptides were purchased from UCB, Bioproducts (Braine l'Alleud. Belgium). The amino-acid sequences are the following with the C-terminus blocked by an amide function:

wild type peptide: GVFVLGFLGFLA-$CONH_2$

vertical mutant: GVFGVALLFLGF-$CONH_2$

The two peptides were dissolved in trifluoroacetic acid. After evaporation of the solvent under N_2 stream and dessication under vacuum, a DMSO solution was prepared (20 μmol peptide/ml DMSO). Cholesterol and phospholipids were purchased from Sigma Chemical Co (St Louis, MO, USA). Multilamellar vesicles (MLV) were prepared in a Tris buffer pH 7.4 containing 15% D_2O. The MLV composition was: dipalmitoyl phosphatidyl choline (DPPC) 27.5 mol %; dipalmitoyl phosphatidyl ethanolamine (DPPE) 27.5 mol %; dipalmitoyl phosphatidyl serine (DPPS) 5 mol % and cholesterol 40 mol %.

Nuclear magnetic resonance. ^{31}P NMR spectra were obtained at 101.3 MHz with a WM 250 Bruker spectrometer. Ten mm tubes were used with 1.5 ml of MLV suspension. FT conditions were: 25 kHz spectral width, 4K data points, flip angle 40° (10 μs), 1.2 s pulse interval. 5000 scans were accumulated and a 50 Hz line broadening was applied to the FID before Fourier Transformation. Powergated ^{1}H decoupling was applied. Experiments were done as a function of temperature: samples were heated from 308 to 348 K and cooled down to 308 K with 30 min equilibration time between each accumulation at a new temperature.

Molecular modeling. The method used to calculate the conformation of the fusion peptide takes into account the variation of the dielectric constant and the transfer energy of atoms from a hydrophobic to a hydrophilic environment that characterizes the lipid-water interface. The structure, mode of insertion, and orientation of the fusion peptide were studied in DPPC monolayer by the method described by Brasseur (4). The procedure used to surround one peptide with lipids in order to generate a monolayer is described in Brasseur

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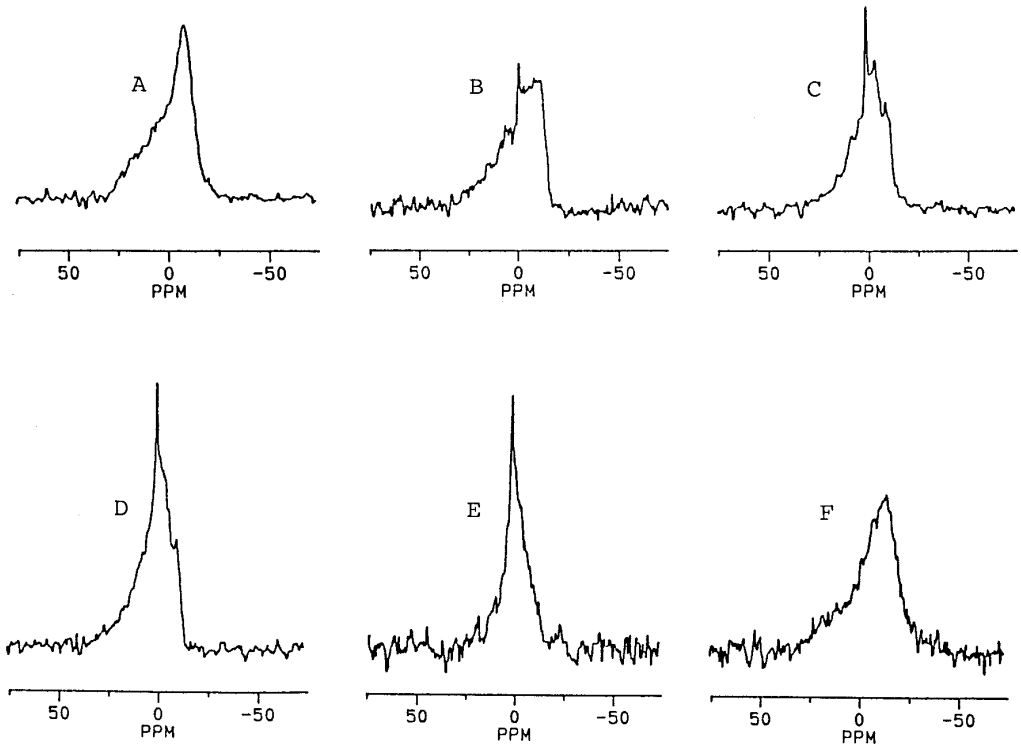

FIG. 1. 101.3 MHz ^{31}P NMR spectra of mixed MLV (DPPC-DPPE-DPPS-Chol, 27.5: 27.5: 5: 40 mol %) at 308K: control liposomes after one temperature cycle (A) ; MLV submitted to the action of wild-type SIV fusion peptide after one temperature cycle for peptide/phospholipid 1/16(B), 1/12(C), and 1/8(D); after two temperature cycles for peptide/phospholipid 1/10(E); MLV submitted to "vertical" mutant (peptide phospholipide 1/5) after two temperature cycles(F).

and Ruysschaert (5). After orientation at the lipid-water interface, the position of the peptide molecule is fixed. The assemblage is completed when the fixed helix is surrounded by the number of first neighbouring lipids. The total conformational energy is calculated as the sum of the contributions resulting from van der Waals energy interactions, torsional potential energy, electrostatic interactions and transfer energy. The total energy is minimized until the lowest energy state of the entire aggregate is reached. To limit the number of possible degrees of structural freedom, bond lengths and bond angles were assumed to be constant. The values are those commonly used in conformational analysis (6). All calculations were performed with PC equipped with Pentium processors using PC-TAMMO+ program (Theoretical Analysis of Molecular Membrane Organization). Graphs were drawn with the Win MGM program (Ab Initio Technology, Obernai, France).

RESULTS

Spectrum A of figure 1 shows the classical "bilayer" spectrum of mixed MLV after one temperature cycle. Its shape after thermal treatment is similar to the shape of the first spectrum obtained at 308 K. Another temperature cycle has no influence on its shape. Spectra B, C, D of figure 1 show the destabilization effect of the SIV fusion peptide on the bilayer organization of phospholipids. The residual powder pattern is perturbed and an isotropic type narrow signal is induced in a dose-dependent manner after successive additions of the peptide. The influence of a supplementary thermal treatment after addition of the wild type peptide is shown by spectrum E. Spectrum F obtained after 2 temperature cycles in the presence of high concentration of the vertical mutant does not show significant shape modification compared to the control liposome spectrum.

The left part of figure 2 shows the oblique mode of insertion of the N-terminal region of the SIV wild type peptide in the lipid layer. This orientation induces lipid destabilization: the parallelism between the acyl chains classically observed in the pure lipid layer is broken because of the peculiar insertion of the peptide. The vertical mutant introduces much less perturbations in the lipid acyl chains as shown in the right part of figure 2.

DISCUSSION

Computer analysis led to the conclusion that fusogenic helices were obliquely oriented with respect to the lipid-water interface. This peculiar insertion was shown to disorganize the lipid layer and is assumed to be involved in the fusion process.

The influence of the two peptides of this study on virus-cell membrane fusion is known. The fusogenic activity of the wild type gp 32 is much higher than that

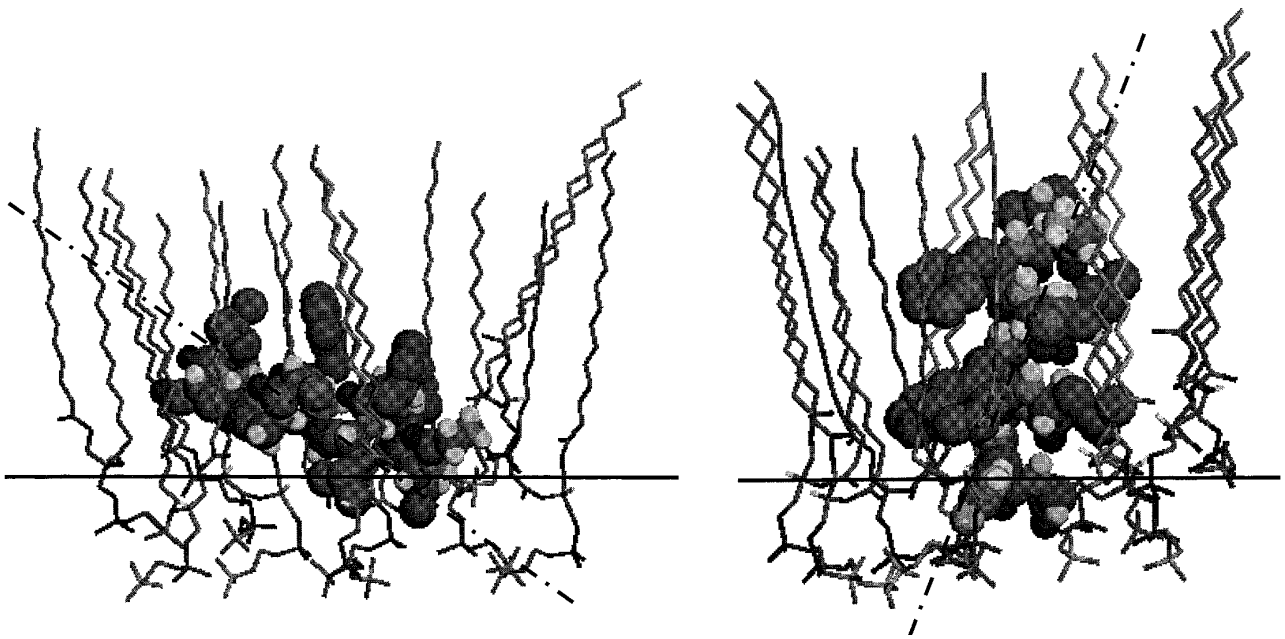

FIG. 2. Mode of interaction of SIV peptides with dipalmitoyl phosphatidyl choline; on the left: wild type peptide; on the right: vertical mutant peptide. Horizontal lines represent the lipid–water interface, and dotted lines represent the axis of the peptides.

of the mutated glycoprotein (3). The reduced fusogenic character of the mutant was attributed to a modification of the mode of insertion into the membrane: the orientation of the mutant peptide is more perpendicular to the lipid-water interface. If the mode of insertion of the peptide in the bilayer is determinant for membrane structure perturbations, ^{31}P NMR is a useful method for determining experimentally the degree of structure modification of phospholipids induced by peptides. Our results confirm this viewpoint. The wild type fusion peptide induces strong perturbations of the bilayer structure: the dose-dependent narrow signal induced by the peptide indicates development of non-bilayer structures or formation of small phospholipid particles. That could be a consequence of the fusion phenomenon. Larger vesicles could be more sensitive to thermal treatment: we have observed that the importance of the narrow signal increases with temperature elevation. It remains difficult to specify the nature of these structural modifications, but development of isotropic ^{31}P NMR signals was also observed with viral fusion proteins (7-8) and fertilin α fusion peptide (9). Moreover, our observations on the influence of a non fusogenic "vertical" mutant of SIV peptide demonstrated that a more vertical insertion of a peptide into the membrane does not induce enough membrane destabilization to be detected by ^{31}P NMR.

This study reinforces the correlation existing between fusion process, formation of structures not organized in extended bilayers and calculated mode of insertion of peptides into lipid layers.

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