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Liposome destabilization induced by synthetic lipopeptides corresponding to envelope and non-structural domains of GBV-C/HGV virus. Conformational requirements for leakage

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Abstract

Liposomes have been used primarily as a model system for studying biological membranes. Numerous chemical, biochemical and biophysical methods have been used to elucidate the various aspects of the interaction between proteins or peptides and phospholipids. Having in mind the potential use of synthetic lipopeptides as antiviral therapies and aiming for a better understanding of the molecular interaction of the GBV-C/HGV with liposomes as model membranes, epitopes of GBV-C/HGV located at the E2 (99-118) and NS3(440-460) regions were selected. Peptides were modified at the N-terminus with acyl chains of different length (C_{14} and C_{16}) yielding the corresponding myristoil and palmytoil lipopeptides. The main aim of the present study was to get insight into the membrane-interacting properties of the above-described synthetic lipopeptides and to study their inhibition of the capacity of perturbing model membranes of fusion peptide of HIV-1 using fluorescence spectroscopy. In an attempt to establish a relationship between peptide membrane activity and structure, we use Circular Dichroism (CD) and Fourier-Transform Infrared Spectroscopy (FTIR).

Keywords: Hepatitis G virus; Lipopeptides; Liposomes; Fluorescence; Circular dichroism; Fourier transformed infrared spectroscopy

1. Introduction

Hepatitis G virus (GBV-C/HGV) is a positive, single-strand RNA virus that has been classified in the family Flaviviridae which is transmitted by contaminated blood and/or blood products, intravenous drug use, from mother to child and sexually. The natural history of GBV-C/HGV infection is at present not fully understood and its potential to cause hepatitis in humans is questionable. Epidemiological studies have indicated that it does not cause acute or chronic hepatitis [1–4]. In recent years numerous studies have been published in which co-infection with GBV-C/HGV and the human immunodeficiency virus (HIV) have been associated with slower progression of the illness and a higher survival rate of patients once AIDS has developed [5–7]. The mechanism responsible for the beneficial effect that the GBV-C/HGV virus has on the course of infection caused by HIV has not been defined.

Elucidation of the mechanism of the fusion of enveloped viruses to target membranes has attracted considerable attention because of its relative simplicity and potential clinical importance. Apart from the functions of viral binding to target membranes and the activation of viral fusion proteins, usually only one viral protein is responsible for the actual membrane fusion step. However, the nature of the interaction of viral fusion proteins with membranes and the mechanism by which these proteins accelerate the formation of membrane fusion intermediates are poorly understood [8]. To simulate protein-mediated fusion, many studies on peptide-induced membrane fusion have been conducted on model membranes such as liposomes and have employed synthetic peptides corresponding to the putative fusion sequences of viral proteins [9–11]. On the other hand and due to the fact that membrane-associated

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peptides often show a remarkable structural behaviour, the conformational study of these sequences is important to get information about their activities [12–15].

Several peptide sequences corresponding to the envelope protein E2 of the HGV/GBV-C were synthesized and studied by our group [16–18] and it was suggested that the peptide sequence E2(279-298) corresponding to the internal region of the E2 protein could be involved in the fusion process of the HGV/GBV-C [19]. This sequence was able, on one hand, to bind with high affinity to negatively charged membranes modifying the biophysical properties of phospholipid model membranes and, on the other hand, to permeabilize efficiently negatively charged vesicles [20]. In our group, the membrane-interacting properties of two epitopes of GBV-C/HGV located, respectively, at the regions (99–118) of the E2 envelope protein and (440–460) of the NS3 non-structural protein and their lipo-derivatives were recently studied [21]. We reported a higher affinity binding of parent and lipophilic envelope E2 peptides than non-structural NS3 peptides to model membranes composed of DPPC [16,22]. Lipopeptides have an increased tendency to insert into membranes compared with the same peptide lacking the lipid moiety. When the lipopeptide attaches to the membrane, it can allow the placement of specific binding sites on a membrane surface, and in addition it can alter the physical properties of the membrane [23]. Epand et al. [24] show that lipogastrins inhibit viral fusion preventing the membrane bilayer from forming highly curved structures required as intermediates in membrane fusion. Recent studies show the inhibiting properties of lipopeptides in influenza [25], hepatitis B [26], and HIV-1 infection [27].

In the present work we endeavoured to gain insight into putative liposome destabilization induced by the NS3 and E2 peptides modified at N-terminus with acyl chains of different length (C_{14} and C_{16}, which are sufficient long to sequester the lipopeptide in the membrane) yielding the corresponding myristoil and palmytoil lipopeptides, and to analyze their role as potential inhibitors of the fusion peptide of HIV-1. First, we have studied their effect on the release of an encapsulated fluorophore, and its putative capacity of inhibiting the gp41 HIV-1 fusion peptide (gp41 FP). Second, the structural requirements of peptides to facilitate the leakage of vesicular contents have been analyzed by circular dichroism and Fourier transform infrared spectroscopy.

2. Materials and methods

2.1. Peptides synthesis

The selection of the sequences belonging to NS3 and E2 proteins of GBV-C/HGV used in this work was based on the amphipathic character (Kyte and Doolitle hydropathy scale) [28] and the accessibility (Janin scale) [29] as previously described [22]. The lipopeptides of NS3 (440–460): AIAYYRG; KDSSIIKDGDLVVC and E2 (99–118): VSWFASTGGRDS-KIDVWSLV studied in this work were obtained by solid-phase methodologies and purified by preparative high performance liquid chromatography, as previously described [16].

The gp41 FP: AVGIGALFLGFLGAAGSTMGAAS was suc-cessfully synthesized in a 100% polyethylenglicol-based resin, the ChemMatrix®, that has proved to be a superior support for the solid-phase synthesis of hydrophobic and highly structures peptides.

The final characterization of synthetic peptides was carried out by analytical HPLC, amino acid analysis and electrospray mass spectrometry.

2.2. Liposomes

L-α-dipalmitoylphosphatidylcholine (DPPC) was purchased from Avanti Polar-Lipids, Inc.and was used without further purification.

Pure DPPC was dissolved in a chloroform/methanol (2:1) mixture and the lipid solution was dried by slow evaporation under a constant flow of nitrogen. The last traces of solvents were removed under vacuum at 50 °C. Large unilamellar vesicles (LUVs) were prepared by hydration of the lipid film with Hepes buffer followed by 10 freeze-thaw cycles [30]. This preparation was extruded 10 times through two 100 nm pore-size polycarbonate filters (Nucleopore, Pleasanton, CA) in a high pressure extruder (Lipex, Biomembranes, Vancouver, Canada) above the transition tempreature of DPPC (50 °C).

For the ANTS (8-aminonaphtalene-1,3,6-trisulfonic acid)-DPX (*N*,*N*′-p-xylenebis(pyridinium bromide)) leakage assay [31] about 15 mg of DPPC were dissolved in a mixture of chloroform and methanol (3:1) that was subsequently removed under a stream of nitrogen. About 1 ml of buffer containing 12.5 mM ANTS and 45 mM of DPX from Molecular Probes (Eugene, OR, USA) and 20 mM NaCl and 5 mM Hepes was added to the dry lipid. The osmolarity of the ANTS/DPX solution was adjusted to be equal to that of the buffer in a cryoscopic osmometer (Fiske One-ten). The suspension was frozen and thawed 10 times to assure maximum entrapment prior to extrusion. The final lipid concentration was 10 mM. A stock solution of LUV of approximately 0.1 μm in diameter was formed by extrusion pressure through Nucleopore polycarbonate membranes. The vesicles were separated from unencapsulated material on Sephadex G-75 (Pharmacia, Uppsala, Sweden), equilibrated with 100 mM NaCl/5 mM Hepes buffer (pH 7.4).

Lipid concentration of liposome suspensions was determined by phosphate analysis [32]. Vesicles size was determined from the measurement of the sample diffusion coefficient by photon correlation spectroscopy.

2.3. ANTS/DPX leakage assay

Dequenching of co-encapsulated ANTS and DPX fluorescence resulting from dilution was measured to assess the leakage of aqueous contents [31]. LUV vesicles containing ANTS/DPX were obtained as described in 2.2. The medium in the cuvettes was continuously stirred to allow the rapid mixing of peptide and vesicles. Fluorescence measurements were performed in a Perkin-Elmer LC50 fluorimeter by setting the ANTS emission at 520 nm as a function of time and the

excitation at 355 nm the slit being 6 nm. A lipid suspension was diluted in buffer to give a final lipid concentration of 100 μM; the fluorescence level of this preparation did not vary with time and was set as 0% leakage. Hundred percentage leakage was the fluorescence value obtained after the addition of 10% (vol/vol) Triton X-100.

Leakage of ANTS-DPX from LUV of DPPC induced by GBV-C synthetic peptides was calculated by using the following Eq. (1):

$$\%\ \text{Leakage} = \left[F_f - F_0\right]/[F_{100} - F_0] \times 100 \quad (1)$$

where F_f is the fluorescence value after adding the peptide, F_0 the initial fluorescence and F_{100} the measured fluorescence after the Triton X-100 treatment.

For the inhibition assay of the leakage activity of the gp41 FP, the concentration of the HIV-1 related peptide was fixed at 1 μM and extents of leakage were measured at the gp41 FP-to-lipid mole ratio of 1:50. GBV-C/HGV lipopeptides were incubated for 30 min in DMSO with the gp41 FP at a mole ratios of 1:1; 1:5; 1:10; 1:15; 1:20 (gp41 FP: lipopeptide) prior to addition to the vesicles.

2.4. Haemolysis assay

Haemolytic activity was determined on rabbit red blood cells obtained immediately before their use. Erythrocytes were washed three times in 30 mM Tris–HCl, 100 mM NaCl, 1 mM EDTA, pH 7.4 buffer and diluted in the same buffer until an initial absorbance at 650 nm of 0.1. Increasing amounts of peptides were added to 1 ml of a suspension of red blood cells solution and the mixture was allowed to react for 45 min at 37 °C with constant shaking to avoid sedimentation. Each sample was centrifuged and the absorbance of the supernatant was measured at 415 nm.

Percentage of haemolysis was calculated using Eq. (2).

$$\%\ \text{Haemolysis} = 100(A_x - A_0)/(A_{100} - A_0) \quad (2)$$

where A_x is the absorbance of the peptide sample, A_0 is the absorbance of a control sample containing buffer but no peptide and A_{100} is the absorbance value obtained by hypotonic lysis with pure water.

2.5. Circular dichroism measurements

CD spectra were recorded on a Jasco J-720 spectropolarimeter (Japan Spectroscopic Company, Tokyo). All measurements were done in 5 mM Hepes buffer, pH 7.4. Cells 0.1 cm in diameter were used and peptide concentration was determined both spectrophotometrically using extinction coefficients of Tyr, Cys and Trp [33] and by quantitative amino acid analysis. Concentration determined by these two independent methods differed from each other by less than 5%. The spectra were measured between 190 and 260 nm using a spectral bandwidth of 1 nm and a scan speed of 10 nm/min.

All measurements were performed at 5 °C and the data were expressed in terms of mean residue ellipticities $[\theta]$ ($\text{degcm}^2\ \text{dmol}^{-1}$). Three scans were accumulated to improve the signal to noise ratio. Before reading the peptide spectra, a blank spectrum of the buffer solution was subtracted.

The percentage of α-helix conformation in the peptides was estimated using the formalism of Chen et al. [34]. This approach assumes that the maximum theoretical ellipticity for a given peptide or protein at 222 nm may be derived from the number of amino acid residues n, and the ellipticity at 222 nm of a helix of infinite length described by Eq. (3).

$$\%\ \alpha\text{–helix} = [\theta]_{222}/[-39,500(1 - (2.75/n))]\ \text{degcm}^2\text{dmol}^{-1} \quad (3)$$

Moreover, K2D, Contin and Lincom–Bramhs by the Dichroweb server at www.cryst.bbk.ac.uk7cdweb programs were used to quantitate experimental CD results [35,36].

2.6. Fourier transform infrared spectroscopy

A Bomem MB-120 Fourier Transform Infrared Spectrometer equipped with DTGS detector and nitrogen gas purge was used for transmittance measurements on filters. Following the methodology previously published [37], FTIR spectra were measured with a resolution of 4 cm^{-1} and 50 scans were taken in order to obtain an appropriate signal to noise ratio without losing signal. Ca_2F flow cell and a window of 100 μm path length spacer were used. Reference spectra of solvents were recorded in the same micro-cells and under identical instrument conditions as the samples spectra. Samples contained about 4 mg peptide/ml in TFE. Difference spectra were obtained by digitally subtracting the solvent spectrum from the peptide spectrum. Fourier self-deconvolution was carried out using a halfwidth of 18 and a resolution enhancement factor or 2.0. To resolve overlapping bands, the spectra were processed using Peakfit v 4.0 software. Second derivative spectra were calculated to identify the positions of the component bands in the spectra. The deconvoluted spectrum was fitted with Gaussian band shapes by an iterative curve fitting procedure until good agreements were achieved between experimental and simulated spectra.

3. Results and discussion

3.1. Assesment of vesicle leakage

Leakage of aqueous contents from cells or vesicles as a result of lysis, fusion or physiological permeability can be detected fluorometrically by using soluble tracers. Assays that detect the solution leakage into the external medium exploit the self-quenching properties of a fluorophore. The ANTS/DPX fluorescence quenching assay has been widely used since it was developed by Smolarsky et al [38] to follow complement-mediated immune lysis.

To further explore the specific interaction of the GBV-C/HGV peptides with phospholipid model membranes, the effect of the

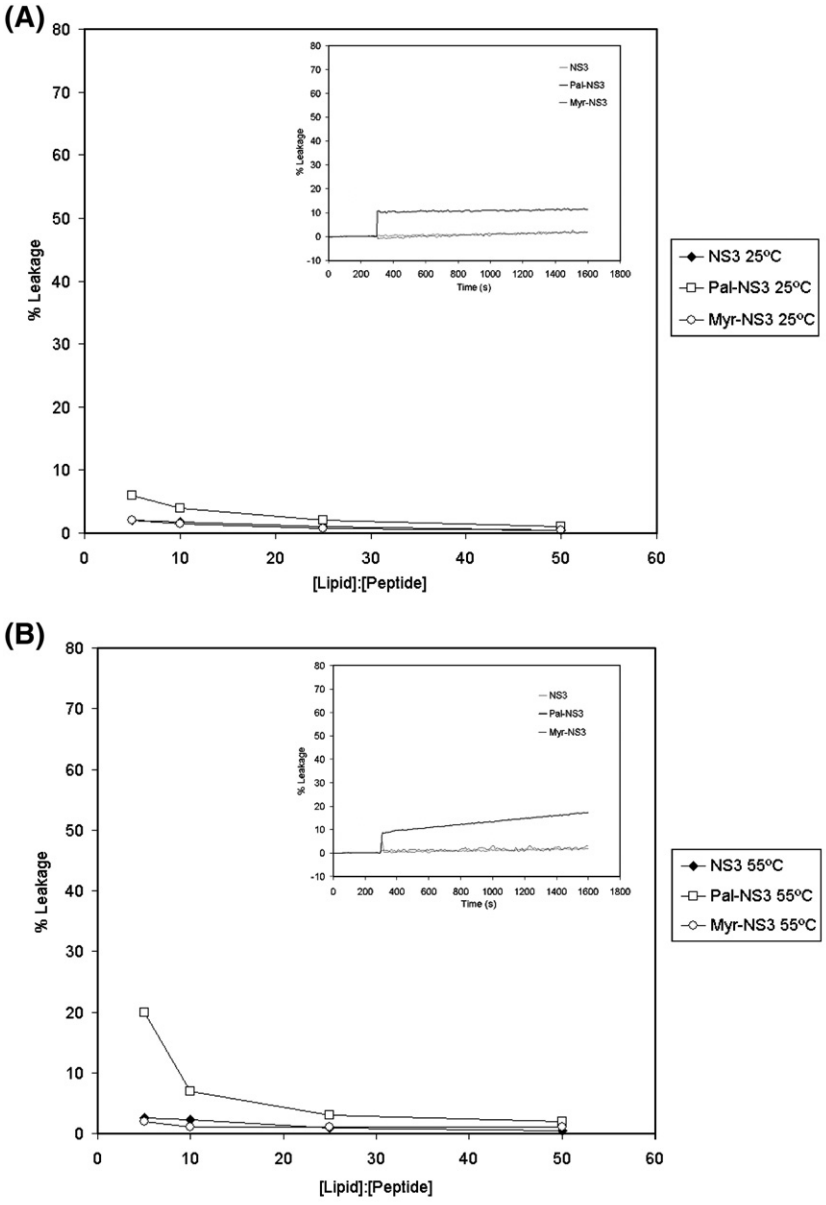

Fig. 1. (A) Leakage of ANTS-DPX from DPPC-LUVs induced by NS3(440–460) peptide and palmitoyl- and myristoyl-NS3 derivatives at different lipid:peptide ratios after 30 min of incubation at 25 °C. Insert: kinetics of leakage induced at lipid-to-peptide ratio of 5. Lipid concentration was 100 μM. (B) Leakage of ANTS-DPX from DPPC-LUVs induced by NS3(440–460) peptide and palmitoyl- and myristoyl-NS3 derivatives at different lipid:peptide ratios after 30 min of incubation at 55 °C. Insert: kinetics of leakage induced at lipid-to-peptide ratio of 5. Lipid concentration was 100 μM.

peptides on the release of the encapsulated fluorophores ANTS/DPX was monitored by dequenching of the ANTS. Previous results have shown that underivatized E2 and NS3 GBV-C/HGV epitopes did not cause any leakage in LUVs-DPPC vesicles at gel phase (room temperature). Above the gel-to-liquid crystalline transition, however, it was clearly observed that whereas the E2 peptide permeabilized vesicles efficiently, the NS3 peptide hardly exerted any effect on DPPC liposomes [22].

Here, in Figs. 1–2, the effect of derivatized E2 and NS3 peptide sequences on the release of the encapsulated fluorophores ANTS/DPX is shown. The time required for the peptides to produce maximum leakage was dependent on peptide concentration. As it can be observed, overall assays carried out with non-structural lipopeptides, Pal-NS3 was able to permeabilize the vesicles at a temperature above the gel-to-liquid transition (Fig. 1B). Nevertheless, the leakage induced by the envelope lipopeptides was clearly higher. On one hand, Pal-E2 and Myr-E2 induced a similar leakage of vesicular contents at room temperature (Fig. 2A). On the other hand, Pal-E2 perturbed lipid bilayers more efficiently at 55 °C achieving values around 45% and 70% corresponding to lipid/peptide molar ratios of 5 and 10, respectively (Fig. 2B). Similarly to described by Plasencia et al [39], the perturbation of phospholipid bilayers induced by the studied peptides affected membrane permeability thus inducing leakage of the aqueous content from DPPC vesicles above the Tm value.

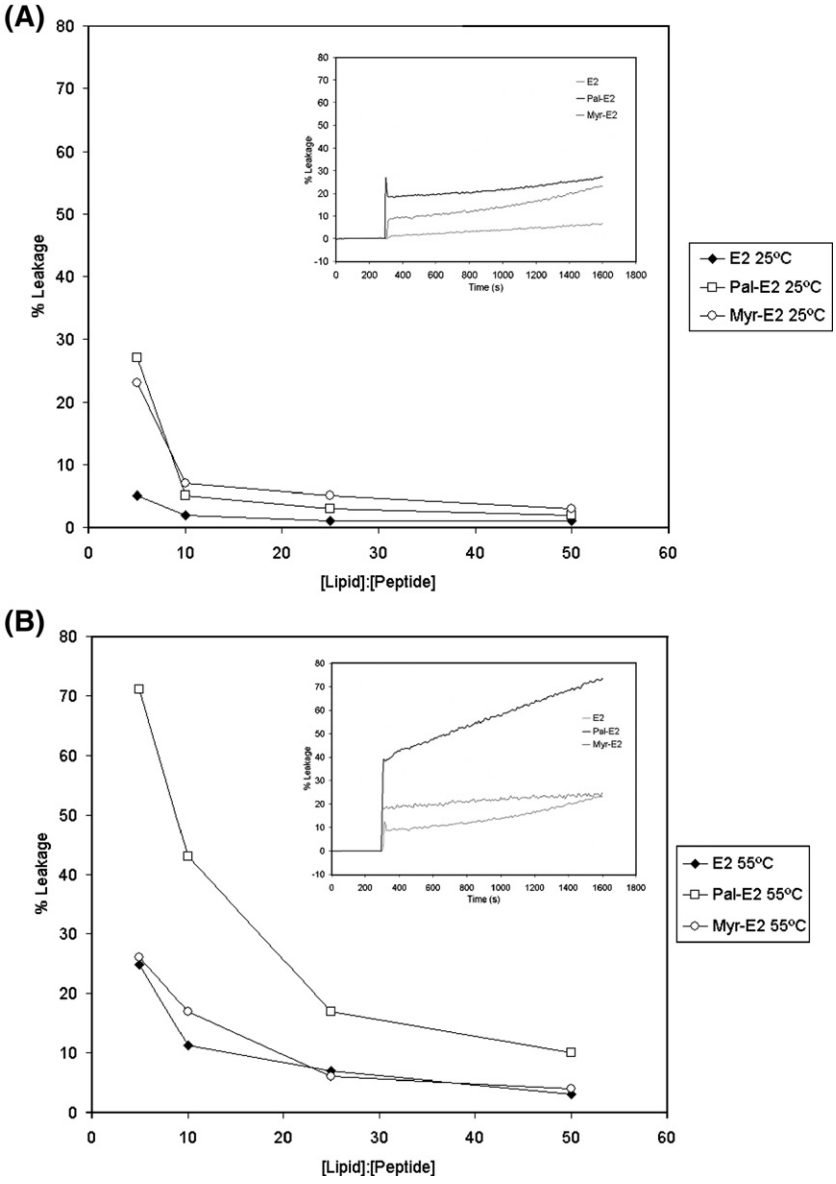

Fig. 2. (A) Leakage of ANTS-DPX from DPPC-LUVs induced by E2(99–118) peptide and palmitoyl- and myristoyl-E2 derivatives at different lipid:peptide ratios after 30 min of incubation at 25 °C. Insert: kinetics of leakage induced at lipid-to-peptide ratio of 5. Lipid concentration was 100 μM. (B) Leakage of ANTS-DPX from DPPC-LUVs induced by E2(99–118) peptide and palmitoyl- and myristoyl-E2 derivatives at different lipid:peptide ratios after 30 min of incubation at 55 °C. Insert: kinetics of leakage induced at lipid-to-peptide ratio of 5. Lipid concentration was 100 μM.

Previous intrinsic emission fluorescence experiments carried out with the peptides in the absence and in the presence of different concentration of DPPC LUVs yielded similar order partition coefficients for E2 and NS3 peptides. Remarkably, the partition isotherms of lipophilic peptides reached saturation at a lower lipid/peptide molar ratio compared to the parent peptides [16]. The results presented here further indicate that Pal-E2 peptide not only interacts with lipid model membranes but also induces perturbations of the lipid packing that extent more deeply into the bilayer.

While the intrinsic lytic effect of PalE2 at room temperature was about 25% (Fig. 2A), the activity of gp41 FP was null or negligible in this type of zwitterionic vesicles (data not shown). Based on these findings and due to the well-documented activity of gp41 FP to permeabilize POPG LUVs, we selected this type of negative vesicles for the study of the ability of lipopeptides of inhibiting the gp41-induced POPG LUV permeability. Not surprisingly, the value of the lipopeptides-induced leakage in this system was considerably higher than the observed in DPPC LUVs. At 1:1 and 1:5 (gp41 FP : lipopeptide) mole ratios the leakage effects were additive achieving values of 52% and 80%, respectively. The 1:10 relationship produced the total leakage of vesicular contents. Consequently, by means of the leakage biophysical assay, no inhibition of the gp41FP activity in POPG model membranes induced by the GBV-C/HGV lipopeptides was observed.

3.2. Haemolytic activity

To further analyze the membrane destabilization properties of the lipophilic E2-peptides, we also studied their haemolytic activity following the experimental procedure described previously in Materials and methods section. Our experimental results showed, on one hand, that the peptides were able to interact with red blood cells as monitored by the increase in A_{415} and, on the other hand, that the haemolytic activity of the studied peptides was dose dependent (data not shown). The results of the haemolysis experiments carried out agree well with the membrane destabilization properties observed for lipophilic derived E2-peptides in the leakage assays previously discussed, achieving the highest percentage of haemolysis values (~40%) for Pal-E2 at 200 μM concentration.

Besides, the ability of the peptide to form transient defects in the bilayer as well as to alter lipid chain packing can be measured by differential scanning calorimetry (DSC). The effects of peptides on the transition enthalpies (ΔH) of the main gel to liquid-crystalline phase transition indicated that the peptides disrupt the packing of the phospholipids, as they interdigitate and cause perturbations that contribute to the bilayer fluidity, with the polar choline head groups not significantly involved. Previously, we have suggested that underivatized peptides are preferentially located at the outer regions of the bilayer, followed by a partial penetration and deformation of the bilayer. Nevertheless, lipophilic derivatives prefer deeper regions and probably a complete penetration into the bilayer [16].

Taken together, the effective disruption of the permeability barrier of the vesicles specifically induced by the Pal-E2 peptide at 55 °C cannot entirely be explained neither by the binding data nor by the effect of the peptide on lipid properties, such as its gel to liquid crystalline phase transition. The conformation of the peptides adopted under functional conditions, i.e. those at which peptide-induced leakage occur, constitute one relevant aspect that has to be considered in the present study. In this sense, structural studies by CD and FTIR spectroscopies were performed in a membranous like environment aiming to better correlate structure and function.

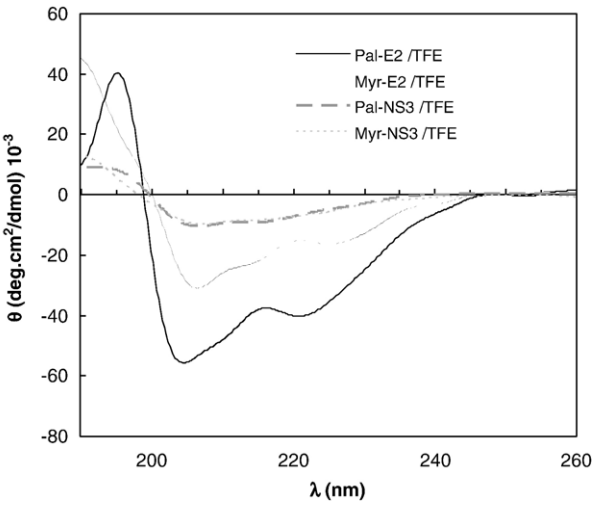

Fig. 3. Circular dichroism spectra of lipopeptides in the presence of 75% TFE.

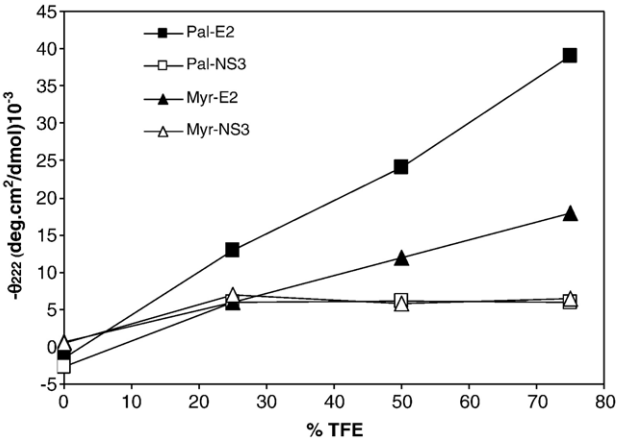

Fig. 4. Molar ellipticity values at 222 nm of lipopeptides after TFE titration.

3.3. CD measurements

The CD spectra of the lipopeptides in Hepes buffer was characterized by a large negative band near 195 nm typical of a random coil conformation (not shown). The addition of TFE clearly contributed to the stabilization of more ordered conformations. As shown in Fig. 3, a maximum at 195 nm and two minima near 208 and 222 nm, characteristic of α-helix structure were observed, the largest contribution of the helical structure being observed for the envelope peptides (Pal- and Myr-E2). In Fig. 4 the dependence of $[\theta]_{222}$ vs. the percentage of halogenated alcohol for the four lipopeptides is shown.

A quantitative analysis of the CD experimental results was performed using K2D, Lincomb–Brahms and Contin programs. In agreement to the results obtained by measurement of the molar ellipticity values at 222 nm the helical content in TFE for Pal-E2 peptide achieved the highest values (Table 1).

Previously, we have shown that neither free peptides nor lipophilic derivatives showed clear CD spectra changes toward more ordered structures in the presence of DPPC vesicles at a lipid-to-peptide ratio of 100:1 [16]. Similar results were obtained below and above the gel/liquid crystalline phase transition temperature of the lipid [16].

3.4. FTIR measurements

FTIR is an established technique for determining the secondary structure of peptides and proteins. The primary information on the peptide conformation is found in the amide

Table 1
Estimation, from the CD spectra, of the content of α-helical conformation of the peptides according to molar ellipticity at 222 nm and different deconvolution computer programs

	% α-helix in 75% TFE			
	θ_{222}	K2D	Lincomb–Brahms	Contin
Myr-NS3	18	20	18	12
Pal-NS3	17	18	26	10
Myr-E2	44	51	47	10
Pal-E2	100	100	92	47

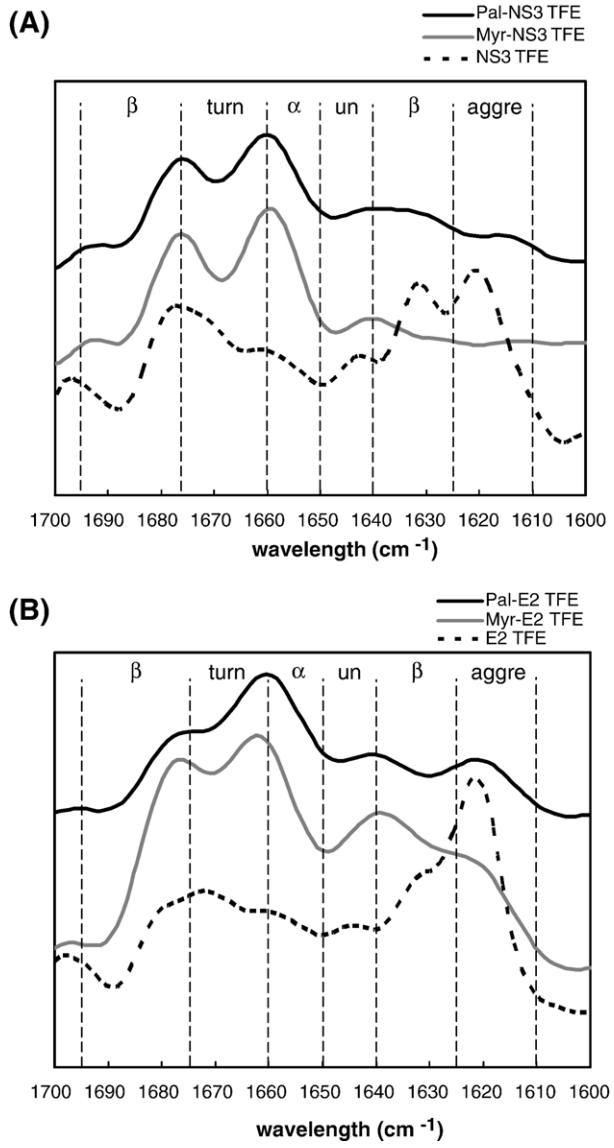

Fig. 5. (A) FTIR deconvoluted spectra of NS3(440–460) and NS3 lipopeptides in 75% TFE for the amide I region. (B) FTIR deconvoluted spectra of E2(99–118) and E2 lipopeptides in 75% TFE for the amide I region.

band. Resolution-enhancement techniques such as deconvolution can be used to identify the positions of components of the amide I band, from which the secondary structures can be identified [40].

FTIR spectra in Fig. 5A corresponding to the NS3 parent peptide show a low-field band splitted into two sharper components centred at 1630 and 1620 cm^{-1}. These bands may be assigned to classical antiparallel β-sheets and intermolecularly H-bonded aggregates of extended structure [41,42]. The conformation sensitive amide I bands of Pal-NS3 and Myr-NS3 in TFE show two maxima at 1660 cm^{-1} and 1675 cm^{-1} revealing a mixture of α-helical and β-turn conformations.

Fig. 5B shows the conformation-sensitive amide I region of the infrared spectrum of the E2 parent peptide and the respective acylated forms in TFE. The spectrum of the E2 peptide in TFE exhibited a maximum absorption at 1625 cm^{-1}, which is related to intermolecular interaction of β-like extended conformations. The presence of a minor component at 1680 cm^{-1} could arise from the high-frequency component of the antiparallel β-structure. This is in good agreement with previous CD results that have shown a negative band with a peak located between 215 and 220 nm, indicating that the organic solvent contributes to preferentially stabilize β-sheet structures [16]. Maximum absorption by acylated peptides, however, was found at 1660 cm^{-1}, indicating that the preferential structure adopted by these peptides is α-helical. It has to be pointed out that the myristoylated peptide shows a higher contribution of unordered (band at 1640 cm^{-1}) and antiparallel β-structures than the palmitoylated peptide. These results show that both Pal- and Myr-NS3 peptides have less ability to adopt α-helical conformation than acylated E2 peptides. It is noteworthy the higher α-helix component for Pal-E2 peptide, the peak at 1660 cm^{-1} being less pronounced for the other lipopeptides.

The perturbation of the lipid bilayer by specific peptide sequences is governed by a subtle equilibrium of electrostatic and hydrophobic interactions [43,44].

Hydrophobic interactions between the acyl chain of the peptides and the lipid acyl chains of the bilayer could be responsible for the higher permeabilizing efficiency. Thus, the activity of the acylated peptides versus their parent peptides could be explained as a function of a number of surface accumulated peptide molecules and their depth of insertion. We have shown previously that palmitoylated and myristoylated peptides penetrate deeper into the bilayer than the underivatized peptides [16]. Nevertheless, there is a significantly different activity between envelope and non-structural peptides that cannot be explained according to hydrophobic interactions and aggregation effects. The conformation of the peptides adopted on the lipid bilayer is essential for their permeabilizing activity. In contrast to acylated NS3 peptides, which maintain an important contribution of β-sheet structure, the acylated E2-peptides, particularly Pal-E2 peptide, adopt mainly a α-helix conformation. This conformation has been proposed to be associated with pore formation by peptides in vesicle membranes, which mediates leakage of low-molecular weight solutes from single vesicles [45–47]. In the pore structure, the peptides are likely to be oriented perpendicular to the plane of the bilayer, resembling the orientation of the transmembrane segments of integral proteins.

Increasing evidence has been obtained that indicates a correlation between helicity of synthetic peptides derived from a wide variety of viral or cellular glycoproteins and their ability to interact with and destabilize a membrane [9].

Our results support the idea that the acyl chains, especially the palmitoyl moiety anchored to the structural E2(99–118) sequence may be able to attach to DPPC vesicles, as recently has been described by others in the context of cell membranes of APCs [48,49] and then the established interaction could cause membrane perturbations leading to the leakage of vesicle-entrapped molecules. The conformational changes from β- to α-structures of E2(99–118) after anchoring the lipidic tails of myristic and palmitic acids agree well with the view that lipopeptides would penetrate membranes as sided insertional helices [50].

With the perspective of gaining further insight into the development of peptide systems resembling closely the membrane-anchored environment of proteins other biophysical studies based on nuclear magnetic resonance, polarized attenuated total reflectance Fourier transform infrared and electron spin resonance spectroscopies could be performed in membrane mimetic environments. These studies will provide useful items of information on peptide-induced changes in the membrane curvature as well as on peptide structures adopted in the lipid bilayer in order to understand how they promote membrane leakage and also could suggest new strategies for the design of GBV-C/HGV peptide inhibitors of the fusion peptide of HIV-1.

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