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Biochimica et Biophysica Acta 1618 (2003) 51–58
BIOCHIMICA ET BIOPHYSICA ACTA
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Interaction of a synthetic peptide corresponding to the N-terminus of canine distemper virus fusion protein with phospholipid vesicles: a biophysical study

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Received 17 June 2003; received in revised form 1 October 2003; accepted 14 October 2003

Abstract

The F protein of canine distemper virus (CDV) is a classic type I glycoprotein formed by two polypeptides, F1 and F2. The N-terminal regions of the F1 polypeptides of CDV, measles virus and other paramyxoviruses present moderate to high homology, supporting the existence of a high conservation within these structures, which emphasises its role in viral-host cell membrane fusion. This N-terminal polypeptide is usually termed the fusion peptide. The fusion peptides of most viral fusion-mediating glycoproteins contain a high proportion of hydrophobic amino acids, which facilitates its insertion into target membranes during fusion. In this work we report on the interaction of a 31-residue synthetic peptide (FP31) corresponding to the N terminus of CDV F1 protein with phospholipid membranes composed of various phospholipids, as studied by means of various biophysical techniques. FTIR investigation of FP31 secondary structure in aqueous medium and in membranes resulted in a major proportion of α-helical structure which increased upon membrane insertion. Differential scanning calorimetry (DSC) showed that the presence of concentrations of FP31 as low as 0.1 mol%, in mixtures with L-α-dimyristoylphosphatidylcholine (DMPC), L-α-dipalmitoylphosphatidylcholine (DPPC) and L-α-distearoylphosphatidylcholine (DSPC), already affected the thermotropic properties of the gel to liquid-crystalline phase transition. In mixtures with the three lipids, increasing the concentration of peptide made the pretransition to disappear, and lowered and broadened the main transition. This effect was slightly stronger as the acyl chain length of the phospholipid grew larger. In the corresponding partial phase diagrams, no immiscibilities or critical points were observed. FTIR showed that incorporation of 1 mol% of peptide in DPPC shifted the antisymmetric and symmetric CH_2 stretching bands to higher values, indicating the existence of an additional disordering of the acyl chain region of the fluid bilayer. FTIR studies of the C=O stretching band indicated that incorporation of FP31 into phosphatidylcholine membranes produced a strong dehydration of the polar part of the bilayer. In mixtures with L-α-dielaidoylphosphatidylethanolamine (DEPE), increasing FP31 concentrations broadened and shifted to lower temperatures the lamellar to hexagonal-H_{II} phase transition, indicating that this peptide destabilized the bilayer and promoted formation of the hexagonal-H_{II} phase. The results are discussed in terms of lipid–peptide hydrophobic mismatch and its influence on the role of the N-terminal polypeptide of CDV F1 protein in viral membrane fusion.
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Keywords: Canine distemper virus; Viral peptide; Phospholipid membrane; DSC; FTIR

1. Introduction

Canine distemper virus (CDV) is an enveloped virus which belongs to the genus Morbillivirus within the family Paramyxoviridae. Morbilliviruses are extremely contagious pathogens and cause some of the most severe diseases in man and animals [1,2]. These viruses infect the host cell upon fusion of its envelope with the cell plasma membrane in a process which is mediated by the viral envelope fusion (F) protein. In the morbilliviruses, the fusion proteins are essential for virus penetration and virus spreading from cell to cell [3,4]. The F protein of CDV is a classic type I glycoprotein [5]. The mature protein is formed by two polypeptides, F1 and F2, which are linked by disulfide bridges [6]. Amino acid sequence analysis has revealed that the N-terminal regions of the F1 polypeptides of CDV, measles virus and other paramyxoviruses present moderate to high homology, supporting the existence of a high conservation within these structures [7], which emphasises its role in viral membrane fusion. This N-terminal polypeptide is usually termed the fusion peptide. The fusion

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doi:10.1016/j.bbamem.2003.10.005

peptides of most viral fusion-mediating glycoproteins contain a high proportion of hydrophobic amino acids, which facilitates its insertion into target membranes during fusion (see Ref. [8] for a review).

The fusion of lipid bilayers is a key step for a number of biological processes of great importance, such as membrane trafficking, fertilization or virus entry. The most extensively studied of these events is by far the membrane fusion of enveloped viruses (see Ref. [9] for a review). It is clear that, when studying viral membrane fusion, there are many areas to be covered. An important field is that concerning the interactions between the viral fusion proteins and the lipids of the target membrane. Synthetic peptides corresponding to defined regions of the fusion proteins offer several unique approaches to study molecular interactions, since the degree of homogeneity is difficult to obtain through purification [10,11]. These peptides have been extensively applied to unravel the molecular characteristics of viral membrane fusion in different systems [12–15]. In this work we report on the interaction of a 31-residue synthetic peptide (FP31) corresponding to the N terminus of CDV F1 protein with phospholipid membranes composed of various phospholipids. Biophysical techniques such us differential scanning calorimetry (DSC), FTIR or X-ray diffraction have been used in order to study the influence of the insertion of FP31 on the physical properties of the target lipid membrane.

2. Materials and methods

2.1. Materials

A 31-residue peptide (FP31) corresponding to the N terminus of the F protein of CDV was synthesised and purified by the Service of Peptide Synthesis of the University of Barcelona (Spain) to the sequence FAGVVLAGVALGVATAAQITAGIALHQSNLN. L-α-Dimyristoylphosphatidylcholine (DMPC), L-α-dipalmitoylphosphatidylcholine (DPPC), L-α-distearoylphosphatidylcholine (DSPC) and L-α-dielaidoylphosphatidylethanolamine (DEPE) were from Avanti Polar Lipids Inc. (Birmingham, AL). All the other reagents were of the highest purity available. Water was twice-distilled in an all-glass apparatus and deionized using a Milli-Q equipment from Millipore (Bedford, MA). A stock solution of FP31 (1 mg/ml) was prepared in toluene with the aid of gentle sonication, whereas the various phospholipids were dissolved in chloroform/methanol (2:1) and stored at −20 °C.

2.2. Vesicle preparation

Vesicles for the DSC and X-ray diffraction measurements were prepared by mixing the appropriate amount of lipids and peptide, as indicated, in organic solvent. The solvent was gently evaporated under a stream of dry N_2, to obtain a thin film at the bottom of a small thick-walled glass tube. Last traces of solvent were removed by a further 2-h dessication under high vacuum. To the dry samples, 1 ml of a buffer containing 100 mM NaCl, 0.1 mM EDTA, 5 mM HEPES pH 7.4 was added, and multilamellar vesicles were formed by vortexing the mixture. Multilamellar vesicles for the FTIR measurements were prepared in the same way in a D_2O buffer containing 100 mM NaCl, 0.1 mM EDTA, 5 mM HEPES pD 7.4.

2.3. DSC

Multilamellar vesicles prepared as described above were pelleted down in a bench microfuge, the supernantants were discarded and the pellets were placed in small aluminium calorimetry pans, sealed and scanned. Scans were carried out in a Perkin-Elmer DSC-7 equipment, at heating rates of 4 °C min^{-1}. A slower rate of 1 °C min^{-1} was occasionally used and it did affect neither the onset and completion temperatures nor the enthalpy of the transitions. The calorimeter was calibrated using pure indium as well as the transition of pure DPPC as standards.

Partial phase diagrams for the lipid component were constructed as previously described [16]. The *solidus* and *fluidus* lines of the diagrams were defined by the onset and completion temperatures of the phase transition on heating scans. In order to avoid artefacts due to the thermal history of the sample, the first scan was never considered. Second and further scans were carried out until a reproducible and reversible pattern was obtained, which usually occurred already with the second scan.

2.4. Infrared spectroscopy

For the infrared measurements, multilamellar vesicles prepared as described above were placed between two CaF_2 windows (25 × 2 mm) separated by 50-μm Teflon spacers and transferred to a thermostated Symta cell mount. Infrared spectra were acquired in a Nicolet MX-1 Fourier-transform infrared spectrometer (FTIR) (Madison, WI), provided with computer data collection. Each spectrum was obtained by collecting 27 interferograms. The D_2O buffer spectrum taken at the same temperature was subtracted interactively using GRAMS/32 (Galactic Industries, Salem, MA), as described previously [17]. This subtraction also compensated the small traces of water that could be present in the samples.

2.5. Small-angle X-ray diffraction

Cu K_α ($\lambda = 1.54$ Å) X-ray was obtained from a Philips (model PW3830) anode. Lipid dispersions, prepared as described above, were measured in a quartz capillary holder, using a Kratky Compact Camera mod. HMB-Graz from H.M. Braun (Graz, Austria) connected to a position sensitive detector (model PSD-50M) from MBraun (Garching, Germany). The sample temperature was kept to ±0.5 °C

using a Peltier system. The system was allowed to equilibrate for about 5 min at each temperature before measurements. Typical X-ray exposure times were 10–15 min for each sample.

For the measurement of lattice spacing, crystalline silver stearate was used as standard. This compound shows a sharp reflection corresponding to a 48.8-Å d-spacing, which is very adequate for calibration. The relative spacing of the peaks in the diffractogram allows specification of the packing symmetry of the phase (lamellar, L or hexagonal-H_{II}, H_{II}) and the distance of the peaks to the center (non-diffracted beam) allows calculation of the repeat or d-spacing of that particular phase.

2.6. Other methods

Phospholipid phosphorous was determined according to the method of Böttcher et al. [18]. Secondary structure prediction of peptide FP31 was performed using Antheprot software.

3. Results and discussion

In order to investigate the perturbations induced in the target membrane upon interaction with CDV fusion protein, a synthetic 31-residue peptide, approximately corresponding to the sequence of the N-terminal end of the viral F1 protein (fusion peptide), was synthesised, and the structure of the peptide as well as its interaction with model membranes were investigated by using a number of relevant physical techniques.

3.1. Solution and membrane structure of FP31 peptide

Examination of FP31 primary structure (see Materials and methods) indicates that the peptide is highly hydrophobic, with only eight, among the 31 residues, being of polar character. Secondary structure prediction in the solid state yielded an 84% of α-helical structure, and a 16% of random coil. Essentially, from residues 1 to 28 the peptide adopts a α-helical structure, whereas the C terminus (residues 29–31) is in a coiled conformation (Fig. 1A). In the helical conformation the polar residues are distributed in both sides of the helix (Fig. 1B), but they concentrate in the portion closer to the C-terminal end, resulting in a extremely hydrophobic N-terminus and a polar C-terminus, giving to the peptide an amphiphilic character. A predominantly helical conformation and an amphiphilic nature are common characteristics of fusion peptides in most fusion glycoproteins of enveloped viruses [10,19].

The secondary structure of FP31 in aqueous medium and in membranes was investigated by using FTIR. Fig. 2 shows the region corresponding to the amide I absorption band of the FTIR spectrum of FP31 in D_2O and in DMPC membranes. At first sight, both spectra are very different suggesting that there are changes in secondary structure. Band fitting of the spectrum in D_2O (panel A) using a mixed Gaussian–Lorentzian function yielded four component bands at 1673, 1648, 1637 and 1624 cm^{-1}, assigned to aggregated strands, α-helix, β-sheet and aggregated strands, respectively [20]. The relative areas of these bands corresponded to approximately a 55% of α-helical structure and a 45% of aggregated strands. Fig. 2B shows the amide I band of FP31 incorporated in DMPC bilayers at 32 °C, i.e. in the fluid state. Band fitting performed as above resulted in a 64% of α-helical structure and a 36% of aggregated strands. This indicated that there is some increase in the proportion of the helical motive upon incorporation of the peptide into phosphatidylcholine membranes since, among different samples, we found an experimental error of ± 2%. These experimental data do not quantitatively coincide with those obtained by computer-aided secondary structure prediction, but qualitatively agree in that FP31 predominantly adopts a α-helical conformation. Furthermore, the data indicate that FP31 aggregates in water solution and in membranes, but the proportion of aggregation decreases when the peptide is incorporated into phospholipid bilayers, probably due to the higher proportion of α-helical structure and increased peptide–lipid interactions.

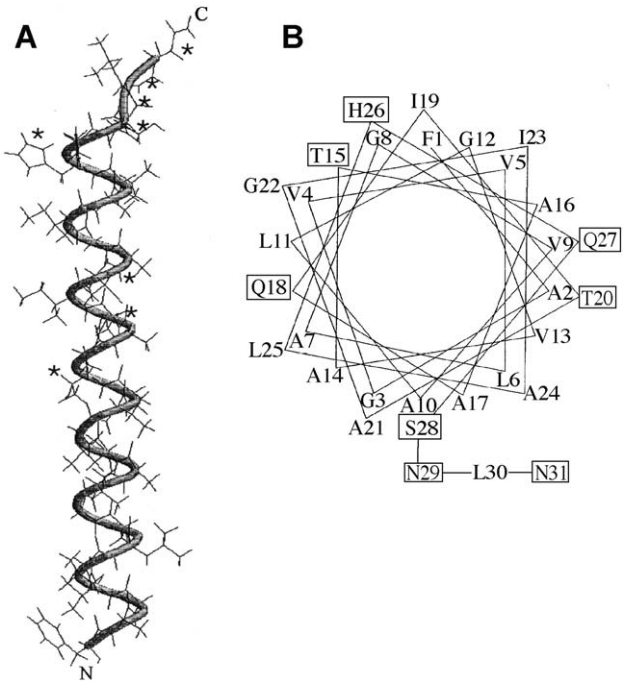

Fig. 1. Structures of FP31. (A) Sketch of the backbone conformation obtained by secondary structure prediction. The asterisks indicate the position of polar residues. (B) Helical wheel projection. The polar residues are boxed.

3.2. Interaction of FP31 with phosphatidylcholine membranes

To obtain information about the interaction of FP31 with phospholipid model membranes, several biophysical tech-

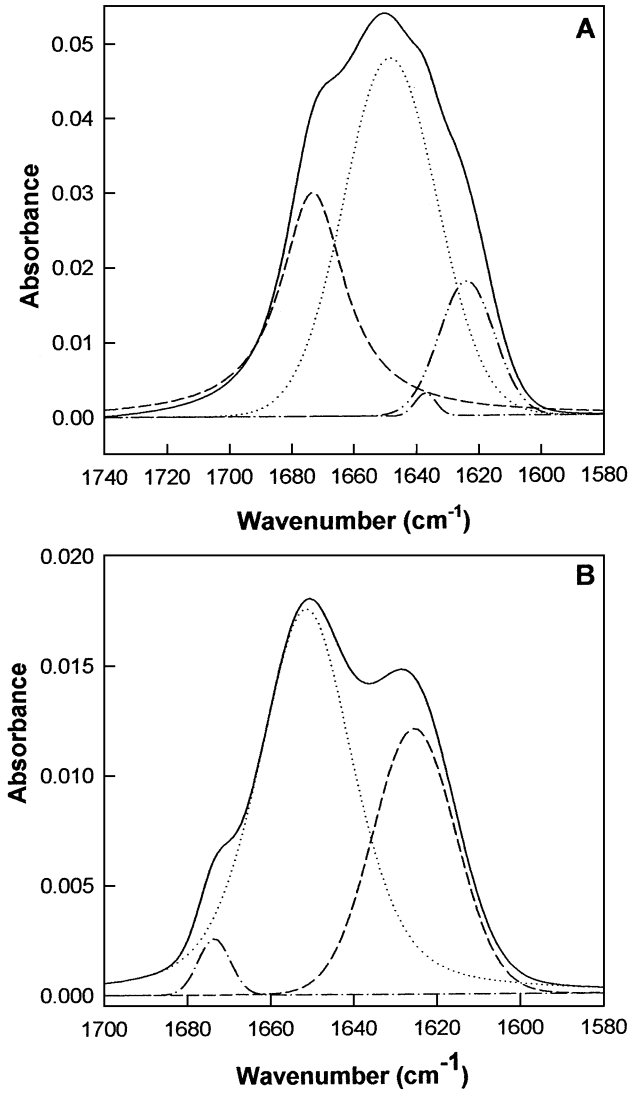

Fig. 2. (A) FTIR spectrum of the amide I absorption of FP31 in D_2O at 25 °C. The solid line corresponds to the original spectrum. The component bands obtained by band fitting are shown: 1673 cm^{-1} (dashed), 1648 cm^{-1} (dotted), 1637 cm^{-1} (dot-dashed) and 1624 cm^{-1} (two dot-dashed). (B) FTIR spectrum of the amide I absorption of FP31 in DMPC bilayers (1 mol%) at 32 °C. The solid line corresponds to the original spectrum. The component bands obtained by band fitting are shown: 1674 cm^{-1} (dot-dashed), 1651 cm^{-1} (dotted) and 1625 cm^{-1} (dashed).

niques were used. Fig. 3 shows the influence of FP31 on the thermotropic gel to liquid-crystalline phase transition of three phosphatidylcholine species (i.e. the same polar head group) with different fatty acyl chain length, namely DMPC, DPPC and DSPC. The incorporation of the peptide into the bilayer was dependent on the acyl chain length of the phospholipid (not shown). Thus, for mixtures with DMPC, FP31 could not be totally incorporated at concentrations above 1 mol%, whereas with DPPC and DSPC concentrations of up to 3 mol% could be reached, suggesting that the peptide, with an approximate length of 42 Å, can be better accommodated into the longer C_{16} and C_{18} chains of DPPC and DSPC. Fig. 3 shows that in the absence of peptide, DMPC exhibited a pretransition at approximately 15 °C and a main gel to liquid-crystalline phase transition at 25.2 °C. Similar DSC scans were obtained for DPPC (transitions at 35 and 41 °C) and DSPC (transitions at 49 and 54 °C). These temperatures are within the range of values reported in the literature for these lipids [21]. The presence of concentrations of FP31 as low as 0.1 mol% already affected the characteristics of the two transitions. Increasing the concentration of peptide made the pretransition to disappear and lowered and broadened the main transition, in mixtures with the three lipids.

Fig. 4 shows the enthalpy change for the gel to liquid-crystalline phase transition for the mixtures of FP31 with the different phosphatidylcholine species discussed above. Increasing the concentration of peptide produced a progressive and significant decrease in ΔH of the transition. Although a straightforward comparison cannot be made, the effect was more pronounced in the mixtures with DSPC. This decrease in ΔH should be the result of the diminished hydrophobic interactions between the phospholipid acyl chains themselves due to intercalation of, and therefore interaction with, FP31.

Using the thermal data from the DSC scans shown in Fig. 3, partial phase diagrams for the phospholipid component were constructed (Fig. 5). Despite quantitative differences, the behaviour found was essentially the same for the three phosphatidylcholine species. Thus, increasing the concentration of peptide produced a decrease in both the *solidus* and *fluidus* lines in a near ideal manner, so that no immiscibilities or critical points were observed. This behaviour indicated that the peptide was homogeneously distributed in the phospholipid bilayer, both in gel and fluid phases. The more pronounced effect in the case of DSPC suggests that peptide–lipid hydrophobic interactions are more favourable in this case, therefore decreasing van der Waals lipid–lipid interactions which results in a lowering of the transition temperature and enthalpy. Certainly, the effect on the *fluidus* line was rather weak which, in accordance with the structure described above, could indicate the tendency of FP31 to self-aggregate and to form some sort of peptide-rich domains within the fluid bilayer.

The perturbations exerted by FP31 in the different parts of the phospholipid molecules were investigated by FTIR. Fig. 6 shows the CH_2 stretching bands of DPPC in the absence and presence of FP31. Incorporation of 1 mol% of peptide shifted the antisymmetric stretching band from 2918.7 to 2920.6 cm^{-1}, whereas the symmetric stretching was displaced from 2850.2 to 2851.2 cm^{-1}. These displacements were similar to those observed in pure DPPC as a consequence of the gel to liquid-crystalline phase transition, indicating that the interaction of the peptide with the phospholipid results in an additional disordering of the acyl chain region of the fluid bilayer. Similar disordering effects have been described for other viral fusion peptides, like SIV fusion peptide [22] or feline leukaemia fusion peptide [23].

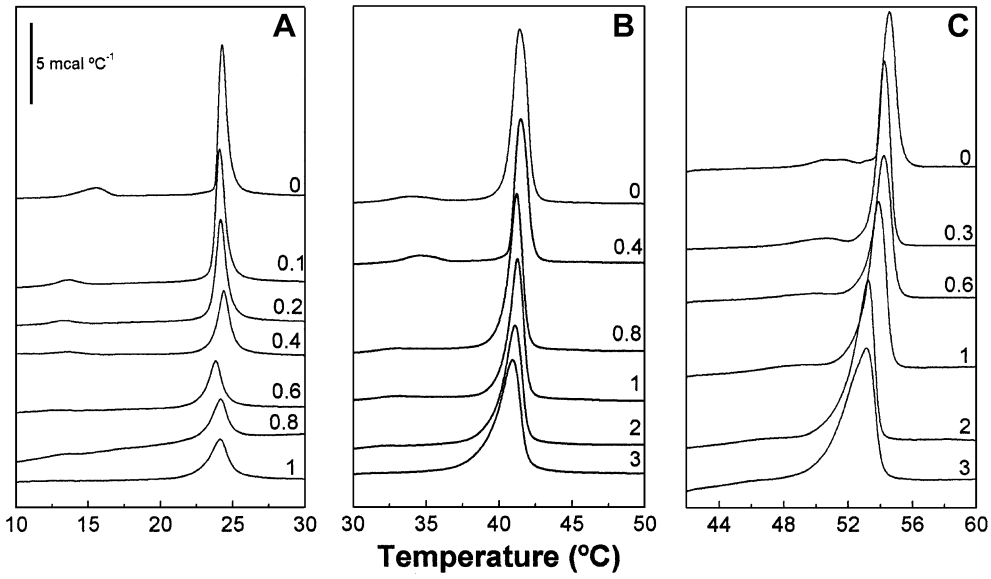

Fig. 3. DSC heating thermograms for mixtures of FP31 with DMPC (A), DPPC (B) and DSPC (C). The concentration of peptide (in mol%) is expressed on the curves. Note that the thermograms are not normalized to the same amount of lipid.

The C=O stretching band of fluid DPPC in the absence and presence of FP31 is shown in Fig. 7. The C=O groups of diacylphospholipids in lipid vesicles may be in a hydrated and a dehydrated state, with their proportion depending on the physical state of the phospholipid bilayer [24,25]. The spectrum of pure DPPC (Fig. 7A) represents a summation of two component bands centered near 1742 and 1727 cm^{-1} (and attributed to dehydrated and hydrated C=O groups, respectively) [26]. The spectra shown in Fig. 7 (solid lines) were subjected to curve fitting using a Gaussian–Lorentzian function. It was obtained that incorporation of 1 mol% of FP31 (Fig. 7B) increased the proportion of the dehydrated component from 18% to 31%, whereas the hydrated one decreased from 82% to 69%, i.e. FP31 interacts with the polar region of the bilayer producing a strong dehydration of the phospholipid head groups. This effect could be one of the main responsible for the fusogenic activity of the N-terminal region of CDV F1 protein. A shift in the maxima of the components of 2 cm^{-1} toward higher values was observed because band fitting was performed allowing a displacement of the maxima of ± 2 cm^{-1}.

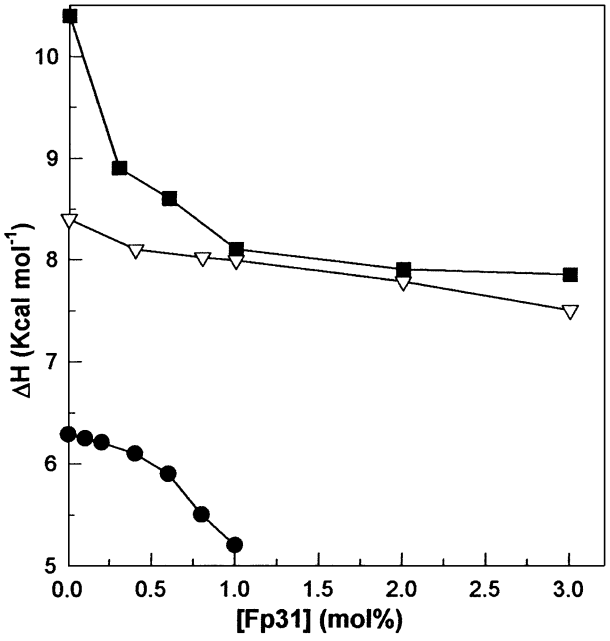

Fig. 4. The enthalpy change for the gel to liquid-crystalline phase transition of mixtures of FP31 with DMPC (circles), DPPC (triangles) and DSPC (squares). The experiment was repeated three times and representative results of one experiment are shown.

Small-angle X-ray diffraction showed that incorporation of FP31 peptide into DPPC membranes did not alter the lamellar organization of this lipid neither below nor above the gel to liquid-crystalline phase transition. Furthermore, the interlamellar repeat distance of 54.8 Å was not modified (results not shown).

As mentioned above, if the helical part of FP31 comprises 28 residues, it should have a length of approximately 42 Å, whereas the hydrophobic core of liquid-crystalline DPPC bilayers should be about 26-Å long [27]. This means that, if most part of this helix inserts perpendicular to the lipid bilayer, there would be a hydrophobic mismatch which will impose a number of energetic constraints. It has been described that there are different possible ways to relieve such constraints: peptide aggregation, peptide tilting, or stretching of phospholipids acyl chains (with the concomitant effect on bilayer thickness) [28]. The weak effect of FP31 on the *fluidus* line of phase diagrams in mixtures with different phosphatidylcholines, together with the presence of aggregated strands as determined by FTIR, suggest that,

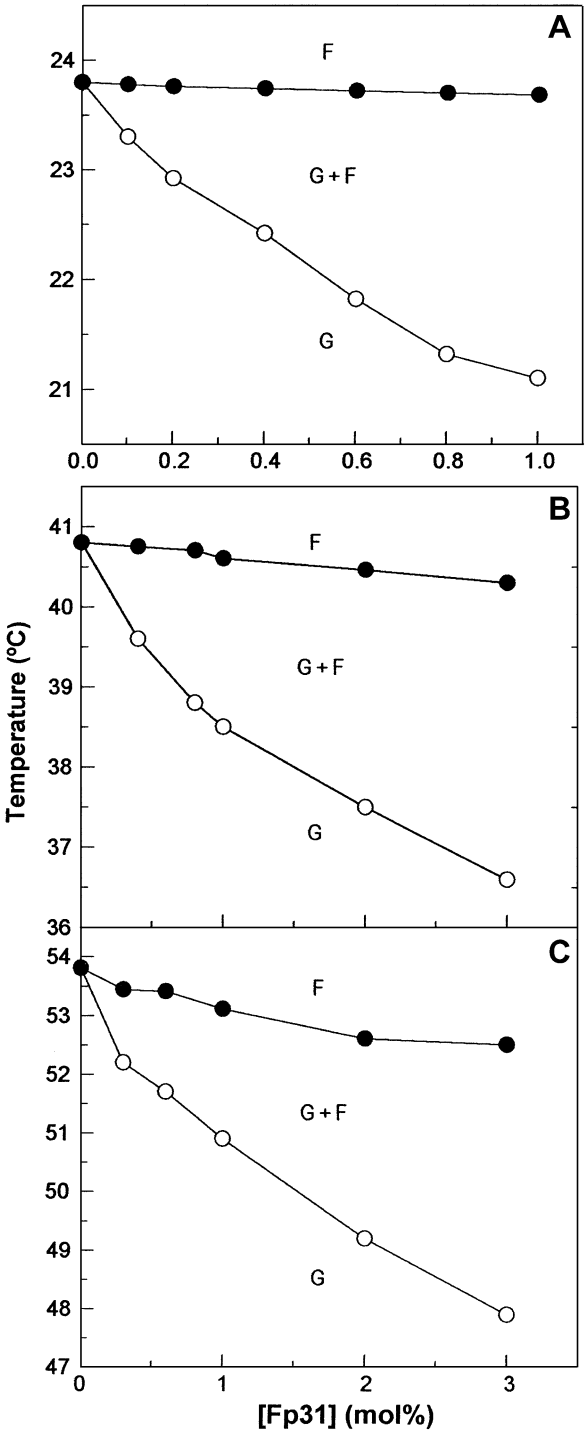

Fig. 5. Partial phase diagrams for DMPC (A), DPPC (B) and DSPC (C) in mixtures with FP31. Open and closed circles were obtained from the onset and completion temperatures of the main gel to liquid-crystalline phase transitions shown in Fig. 4, and represent the *solidus* and *fluidus* lines, respectively. G = gel phase, F = liquid-crystalline phase.

upon incorporation into phospholipid membranes, FP31 presents a high degree of aggregation in order to minimize the exposed hydrophobic area and thus to decrease the mismatch effect. In fact, CDV fusion protein and other viral

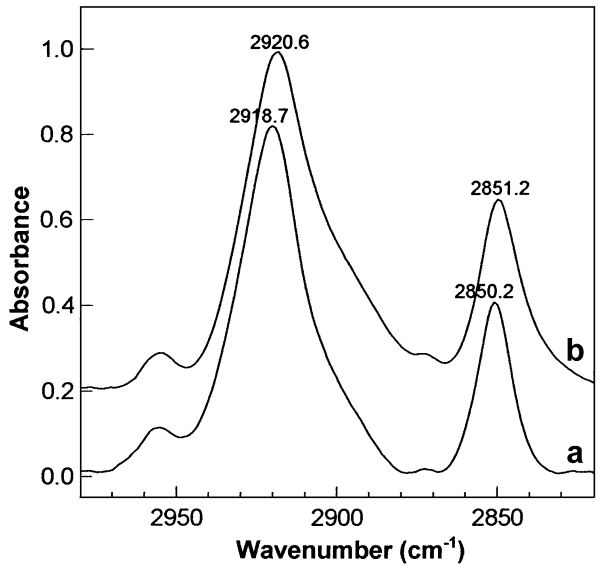

Fig. 6. FTIR spectra of the CH_2 stretching absorption band of DPPC (a) and DPPC containing 1 mol% of FP31 (b) at 50 °C. The frequencies of the maxima are indicated on the bands.

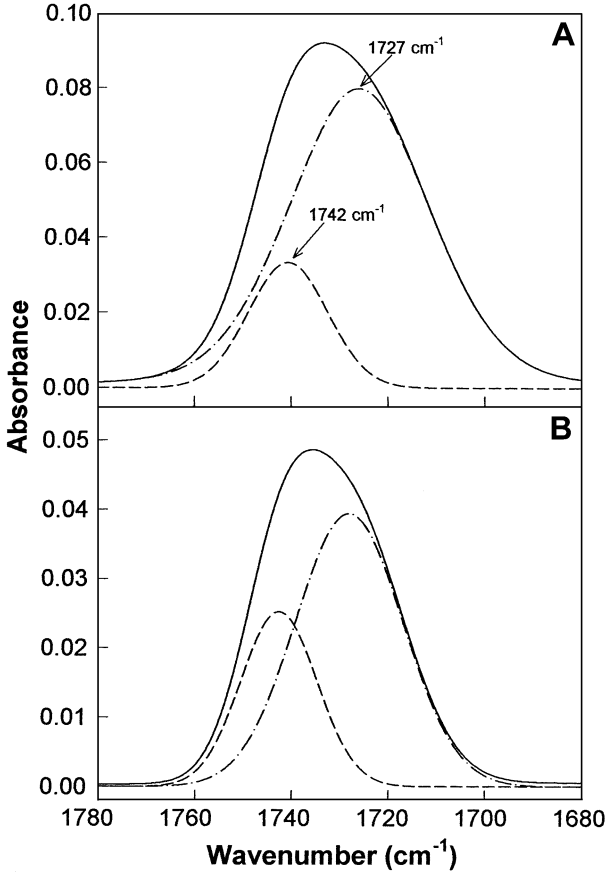

Fig. 7. FTIR spectra of the C=O stretching band of DPPC (A) and DPPC containing 1 mol% FP31 (B) at 50 °C. The dashed lines represent the dehydrated component, and the dot-dashed lines the hydrated component obtained by band fitting.

fusion proteins do not insert fusion peptides into the target membrane as monomers, but there is oligomer formation upon membrane interaction [9]. Thus, influenza virus HA undergoes trimerization to produce membrane fusion [29], and in the case of Sendai virus the viral fusion proteins accumulates at the site of fusion [30]. On the other hand, it should be considered that peptide tilting could also be explained by the sidedness of the helix, as suggested before [31]. Thus, from the helical wheel shown in Fig. 1, it can be seen that a face of the helix has a cluster of amino acids with small side chains (residues 7, 14, 3, 21, 10, 17 and 24), whereas all of the bulky valine residues are on the opposite side of the helical wheel.

It seems that, in addition, there are effects on lipid structure and organization, since the frequency of the CH_2 antisymmetric and symmetric stretching bands is shifted to higher values. This effect could be simply interpreted in terms of conformational order, i.e. the lipid acyl chains in the fluid state are disordered as a consequence of their tendency to adapt to the higher length of the peptide [32]. However, this would also change d-spacing, and we have shown by means of SAXD that this parameter is not affected. We believe that other phenomena such as interchain coupling can induce shifts in the frequency of the methylene stretching bands [33]. Furthermore, the amino side chains of the peptide also contribute to the CH_2 stretching bands of the lipid, producing frequency displacements.

Therefore, in our case, most probably peptide tilting is taking place in order to compensate for the hydrophobic mismatch between the peptide and the hydrophobic core of the bilayer. Thus, aggregates of FP31 would insert in an oblique manner into the target membrane, so that the hydrophilic C-terminal end states close to the polar phospholipid head groups. Interaction of this region with the C=O groups decreases hydrogen bonding, resulting in strong dehydration of the polar part of the bilayer. Membrane dehydration seems to be a requisite to allow close interbilayer contact in early stages of membrane fusion.

3.3. Effect of FP31 peptide on lipid polymorphism

The modulation of lipid polymorphism by FP31 was studied in DEPE systems. Incorporation of the peptide into DEPE produced a weak effect on the main gel to liquid-crystalline phase transition of DEPE, slightly decreasing the transition temperature (not shown). However, FP31 did affect the lamellar fluid to hexagonal-H_{II} phase transition of this phospholipid (Fig. 8). Up to 2 mol% FP31 broadened and shifted to lower temperatures this transition, with a concomitant decrease of ΔH (not shown), indicating that this peptide promotes formation of the hexagonal-H_{II} phase. Other viral peptides, like feline leukaemia fusion peptide [23] or SIV fusion peptide [22], have been also shown to destabilize the lamellar phase and to promote H_{II} formation in phosphatidylethanolamine systems. This bilayer destabilizing (or H_{II} phase promoting) effect can be explained in terms of its oblique mode of insertion into the membrane. Insertion at an oblique angle would expand the center of the bilayer more than the bilayer surface, increasing negative curvature and destabilizing the bilayer in relation to inverted phases [31]. Our results fit well within this explanation. Lipids participate actively in membrane fusion processes. According to the stalk-pore model [34,35], an increased negative curvature in the bilayer would facilitate this process. Our results suggest that the negative curvature strain induced by FP31 may result in fusion promotion as shown before for other peptides [22].

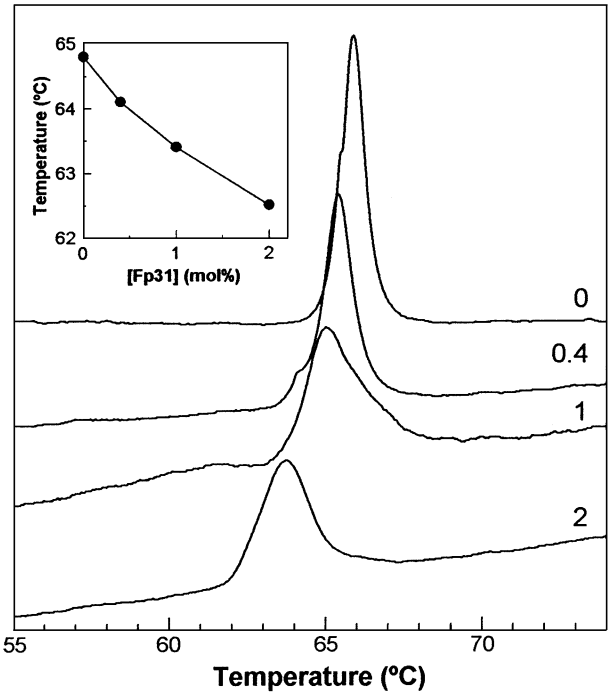

Fig. 8. DSC heating thermograms for mixtures of FP31 with DEPE in the region of the lamellar to inverted hexagonal-H_{II} phase transition. The concentration of peptide (in mol%) is expressed on the curves. Inset: dependence of the lamellar to inverted hexagonal-H_{II} phase transition onset temperature with FP31 concentration. Note that the thermograms are not normalized to the same amount of lipid.

3.3.1. Concluding remarks

Taken together, our results contribute to explain the fusogenic activity of the N-terminal end of CDV F1 protein by a number of facts: (i) adoption of a strongly hydrophobic α-helical structure with a hydrophobic mismatch with the phospholipid acyl chains of the target membrane; (ii) peptide aggregation, insertion into the target membrane at an oblique angle (peptide tilting) and dehydration of the polar part of the bilayer. These data fit well within the currently accepted stalk-pore model of membrane fusion [35] and agree with proposed models for protein-driven viral membrane fusion [9].

Acknowledgements

This work was supported by Fondo de Investigación Sanitaria, Ministerio de Sanidad y Consumo, Spain (Project 01/0136).

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