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Interaction of three β-interferon domains with liposomes and monolayers as model membranes

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Abstract

The physicochemical properties of three peptides belonging to the β-interferon (β-IFN) molecule, -β-IFN(13–20), β-IFN(40–47) and β-IFN(109–116)-, which have been described to be antigenic epitopes of the neutralising antibodies responsible of the failure of the Multiple Sclerosis therapy, and their palmitoylated derivatives were analysed. Peptides were synthesised by solid-phase methodologies and characterized by amino acid analysis, analytical high-performance liquid chromatography and electrospray mass spectrometry. The activity of free and derivatized peptides was determined. In order to know how the synthesised peptides were able to interact with membrane models, studies of kinetics of penetration at constant area and compression isotherms were carried out. Moreover, differential scanning calorimetry (DSC) was used to investigate the thermotropic phase properties of binary mixtures of dipalmitoylphosphatidylcholine (DPPC) or dipalmitoylphosphatidylglicerol (DPPG) with the peptides.

Keywords: β-Interferon; Synthetic peptides; Phospholipids; Monolayers; Liposomes; Differential scanning calorimetry

1. Introduction

Multiple Sclerosis is an autoimmune disease associated with immune activity directed against central nervous system antigens. The most effective treatment available is β-interferon (β-IFN), as a result of its immunomodulatory effect. β-IFN decreases the frequency of relapses and the number of lesions in magnetic resonance imaging as well as it causes a slow disease progression [1]. However, patients treated with β-IFN develop neutralising antibodies against the drug, this fact being a failure of β-IFN therapy [2].

Previous studies have indicated that three main antigenic epitopes on the β-IFN molecule do exist. The molecule of β-IFN contains five α-helices: A (residues 2–22), B (residues 51–71), C (residues 80–107), D (residues 118–136), and E (residues 139–162). These helices are connected by loops designated AB, BC, CD and DE [3]. Mapping the locations of the major observed linear epitopes onto the three-dimensional structure of β-IFN have resulted that most antibodies detected in the ELISA analysis recognise regions of the molecule close the N-terminus, the AB or CD loops, or D helix. These regions possess an extended linear structure in the native, folded molecule [4]. Consequently, antibodies that bind to these regions of the native structure would also bind to linear peptides of the same sequence.

Having in mind that synthetic peptides have been shown to be a valuable tool to mimic the action at the lipid membrane level [5,6], the main aim of the present study was to get insight into the interaction of three putative antigenic synthesised peptides belonging to (13–20), (40–47) and (109–116) portions of β-IFN molecule and its derived lipopeptides with lipid bilayers.

Phosphatidylcholines (PC) are the most abundant lipids in mammalian membranes and a major membrane component in eukaryotic organism. Dipalmitoylphosphatidylcholine (DPPC) has a well-defined transition temperature that facilitates the study of the effect of peptides in its thermotropic properties.

The amphipatic character of these peptides could make them surface active products and as their biological activity

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occurs at lipid membrane interfaces, the monolayer technique is entirely suitable to study their physicochemical and biological properties [7]. Interactions of molecules, in our case peptides, with a bilayer-ordered structure can influence vesicle transition thermotropic parameters according to their own physicochemical properties [8,9]. In this sense, we have used the differential scanning calorimetry (DSC) technique to analyse the effect of increasing amounts of the β-IFN belonging peptides on the thermotropic properties of multilamellar vesicles composed of the zwitterionic lipid DPPC. Moreover, we have studied the effect of 10% of β-IFN lipopeptides with an anionic lipid such as dipalmitoylphosphatidylglicerol (DPPG).

2. Experimental section

2.1. Materials

DPPC and DPPG were obtained from Sigma. Chloroform, methanol and acetonitrile solvents were from Merck. Dimethylformamide (DMF) was purchased from Sharlau. Water was double distilled and deionized (Milli-Q system, Millipore) The resistivity of water was 18.2 MΩ cm and pH was 5.8. Rink Amide MBHA resin and amino acids were obtained from Novabiochem. Coupling reagents were obtained from Fluka and Novabiochem. Trifluoroacetic acid (TFA) was supplied by Merck and scavengers such as ethanedithiol (EDT) or triisopropylsilane (TIS) were from Sigma-Aldrich.

2.2. Methods

2.2.1. β-IFN peptides syntheses

The studied peptide sequences were chosen according to the semiempirical method of Chou and Fasman [10] that theoretically predicts the secondary structure of peptides. This method was applied by using the Peptide Companion version 1.24 (Coshisoft/Peptide Search) computer program. Then, the peptides were synthesised by a solid-phase methodology following an Fmoc/tBut strategy. β-IFN(13–20) (SNFQCQKL), β-IFN(40–47) (IPEEIKQL) and β-IFN(109–116) (EDFTRGAL) were obtained manually on a Rink Amide MBHA (functionalization 0.65 meq/g) by means of a diisopropylcarbodiimide/hydroxybenzotriazole (DIPCD/HOBt) activation. Threefold molar excesses of Fmoc-amino acids were used throughout the synthesis, the yield of each coupling being at least 95% according to Kaiser et al.'s [11] test. At the completion of the introduction of Fmoc-Leu, the peptide resin was removed from the reaction column, washed with DMF, isopropyl alcohol, and ether, dried in vacuum, and divided into three parts. Each part of the amino resin was elongated in order to obtain the about-described peptides. During the synthetic process carried out to obtain the β-IFN(109–116) peptide, repeated couplings for the incorporation of Thr^{112} and Arg^{113} were needed. Final deprotection and cleavage of peptides from the resin was achieved by an acid treatment with TFA containing appropriate scavengers (H_2O, EDT and TIS) at room temperature for about 2 h with occasional agitation. The crude peptides were then precipitated with diethyl ether, the samples were sonicated and centrifuged, and the supernatants were decanted off. This last step was repeated until the total removal of scavengers. Finally, the peptides were dissolved in water and lyophylized.

The synthesised peptides were successfully characterised by analytical HPLC, amino acid analysis and Maldi-Tof mass spectrometry (Table 1).

HPLC analyses were performed on a C-18 silica column eluted with acetonitrile (A)/water (W) (0.05% TFA) mixtures. Conditions used for the three deprotected peptide sequences, were 30 min by a gradient from 85%W to 65%W. Eluted substances were detected spectrophotometrically at 215 nm. A single peak for β-IFN(13–20), β-IFN(40–47) and β-IFN(109–116) with capacity factor values (K') of 6.6, 12.6 and 12.0 respectively, were obtained.

Satisfactory amino acid analyses were obtained. The analyses were carried out in a Pico-Tag system (Waters). Samples of 1 mg of the peptides were hydrolysed in 6 N HCl at 110 °C over 24 h.

2.2.2. Lipopeptides syntheses

Dry peptide resins were swollen in DMF for 30 min and the solvent decanted off. Fmoc protecting groups were removed from protected peptide resins, and palmitic acid was attached to the N-terminus as follows. Palmitic acid was dissolved in a minimum amount of DMF, followed by the addition of DIPCD/HOBT reagents. The solutions were then

Table 1
Peptides characterization

Peptide	aaa[a]	HPLC (K')[b]	Maldi-Tof[c]
β-IFN(13–20): SNFQCQKL	L=1.01 (1), K=1.17 (1), Q=1.98 (2), C=n.d. (1), F=0.75 (1), N=0.84 (1), S=0.84 (1)	6.6	965.9
β-IFN(40–47): IPEEIKQL	L=1.05 (1), $E+Q$=3.23 (3), K=1.21 (1), I=1.51 (2), P=1.07 (1)	12.6	968.0
β-IFN(109–116): EDFTRGAL	L=1.03 (1), A=1.06 (1), G=1.05 (1), R=0.96 (1), T=1.03 (1), F=0.9 (1), D=0.97 (1), E=1.03 (1)	12.0	908.0

[a] Amino acid analysis (theoretical values in parenthesis).
[b] Eluents: (A) H_2O (0.05% TFA), (B) CH_3CN (0.05% TFA); gradient: 85% A to 65% A in 30 min; Detection: λ=215 nm; flow: 1ml/min.
[c] MALDI-TOF mass spectrometry.

poured into the soaked peptide resins. The mixtures were set aside at room temperature and agitated occasionally. Reactions were completed in 2 h as judged by the Kaiser's test. Afterwards, they were rinsed and dried. Finally, palmitoylated peptides were removed from the resins and their identity was confirmed by mass spectrometry.

2.2.3. Monolayer studies

The experiments were performed on a Langmuir film balance KSV5000, equipped with a Wilhemy platinum plate.

2.2.3.1. Surface activity. Surface activity measurements were carried out in a cylindrical trough (volume 70 ml, 30 cm^2) with mechanical stirring. The trough was filled with phosphate-buffered saline (PBS) and increasing volumes of concentrated peptide solutions were injected directly underneath trough a lateral hole. Pressure increases were recorded continuously for 60 min.

2.2.3.2. Insertion of peptides into monolayers. The same methodology was used in the presence of phospholipid monolayers. Monolayers were formed spreading the phospholipids from a 1 mg/ml stock solution in chloroform, directly to the air–water interface, to reach the required initial surface pressure: 5, 10, 20 and 32 mN/m. After pressure stabilisation peptide solution was injected directly underneath the monolayer [12].

2.2.3.3. Compression isotherms. Compression isotherms of peptides or DPPC spread on the aqueous subphase containing peptides were carried in a Teflon trough (surface area 17,000 mm^2, volume 1000 ml) containing 850 ml of PBS. The surface pressure of the monolayers was measured by a Wilhelmy plate pressure sensor and it was calibrated periodically with the π–A curve of the stearic acid monolayer. The uncertainty in the area per molecule obtained from the isotherms was about $\pm$ 5%. Films were spread from chloroform/methanol solutions and at least 10 min was allowed for solvent evaporation. The monolayer was compressed with an area reduction rate of 20 mm^2/min and was compressed up to their collapse pressure. Stability of the monolayers was assessed by compressing them and stopping the barrier at different pressures, and observing that no pressure decay occurred after 30 min. All experiments were performed at 21 $\pm$ 1 °C. Each run was repeated three times.

2.2.4. Differential scanning calorimetry

Multilamellar vesicles (MLVs) of DPPC or DPPG were prepared as follows. Briefly, the lipid was dissolved in a glass tube with a mixture of chloroform/methanol (2/1 v/v) and dried by slow evaporation under constant flow of nitrogen. The residual solvent was removed by storing the samples overnight under high vacuum in a vacuum oven at room temperature. MLVs were obtained by hydrating the lipid film with HEPES buffer, pH 7.4 alone or containing the different peptides at increasing proportions (0%, 3%, 5%, 10%, 20% and 30 %) and vortexing at 50 °C. Final lipid concentration was quantified by phosphorous analysis [13] and was around 4 mM.

DSC experiments of MLVs were performed using a DSC 821E Mettler Toledo (Greifensee, Switzerland) calorimeter. Hermetically sealed aluminium references and samples containing pans were used. Sample pans were loaded by adding 30 μl of DPPC vesicle suspension, corresponding to approximately 0.13 mg of phospholipid. Differences in the heat capacity between the sample and the reference cell were obtained by raising the temperature at a constant rate of 5 °C min^{-1} over a range from 0 to 60 °C. All samples were submitted to three heating/cooling cycles. Data from the first scan was always discarded to avoid mixing artefacts. The endothermic peak coming from the second scan of the control sample was used as a reference template. The calorimeter was calibrated with Indium. To ensure scan-to-

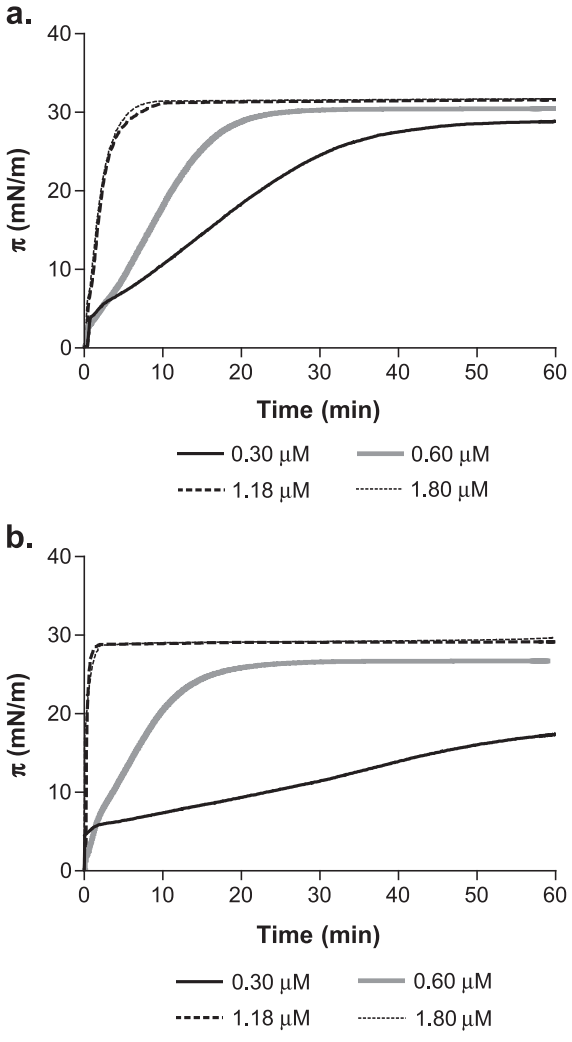

Fig. 1. Surface activity of (a) Palm-β-IFN(40–47) and (b) Palm-β-IFN(109–116) at 0.3, 0.6, 1.18 and 1.8 μM concentrations.

scan reproducibility three consecutive scans of the same sample were performed. DSC runs were carried out within the same day of liposome preparation. Molar enthalpies of transition (ΔH) were calculated from peak areas by means of a STARTe Mettler Toledo system.

3. Results and discussion

3.1. Peptides characterization

The synthesis of free peptides -β-IFN(13–20), β-IFN(40–47), β-IFN(109–116)-, and their derived lipopeptides, -Palm-β-IFN(13–20), Palm-β-IFN(40–47), Palm-β-IFN(109–116)-, was accomplished by using Fmoc/tBu strategy, as described in the experimental section. As shown in Table 1, the synthesised peptides were well characterised by analytical HPLC, amino acid analysis and Maldi-Tof mass spectrometry. Analytical HPLC results indicated that the β-IFN(13–20) peptide sequence is clearly more hydrophilic than β-IFN(40–47) and β-IFN(109–116) peptides, being its K' value two times lower. The mass spectrometry analysis for the Palm-β-IFN(13–20), Palm-β-IFN(40–47), Palm-β-IFN(109–116) confirmed that the syntheses were successfully carried out.

3.2. Surface activity

The surface activities of the peptides were determined by injecting different peptide concentrations into the PBS-buffered surface and recording the surface pressures, π, that were achieved. The experimental curves were used to determine the peptide concentration to be employed in the kinetics of penetration experiments. The chosen concentration, 0.6 μM, was slightly lower than the saturation concentration.

β-IFN(13–20), β-IFN(40–47) and β-IFN(109–116) did not present surface activity as a consequence of their solubilization in the subphase. Due to the high hydrophilicity of β-IFN(13–20) sequence, that has five polar amino acids at the N-terminus, its lipophilicaly derivative neither presented surface activity. Contrarily, the adsorption of Palm-β-IFN(40–47) and Palm-β-IFN(109–116) peptides into the interface was gradual at low concentrations. However, the adsorption at saturation concentrations was achieved very quickly and before 5 min (Fig. 1a and b).

3.3. Penetration kinetics at constant area

The interaction of the peptides that had surface activity with monolayers composed of a zwitterionic phospholipid (DPPC) was studied trough penetration kinetics at constant area at different initial surface pressure: 5, 10, 20 and 32 mN/m. At 32 mN/m, there was not an increase in the pressure when the peptide was incorporated. Peptide concentration in the subphase was 0.6 μM. Fig. 2 shows the pressure change versus time. The maximum pressures were achieved at around 25 min for all initial pressures assayed. The inset of the figure shows the values obtained after 1 h at different initial pressures. In this diagram is shown the effect of the initial surface pressure in the increase induced for the peptides. The greater was the initial pressure the lower was the effect of the peptide. This is a common behaviour for

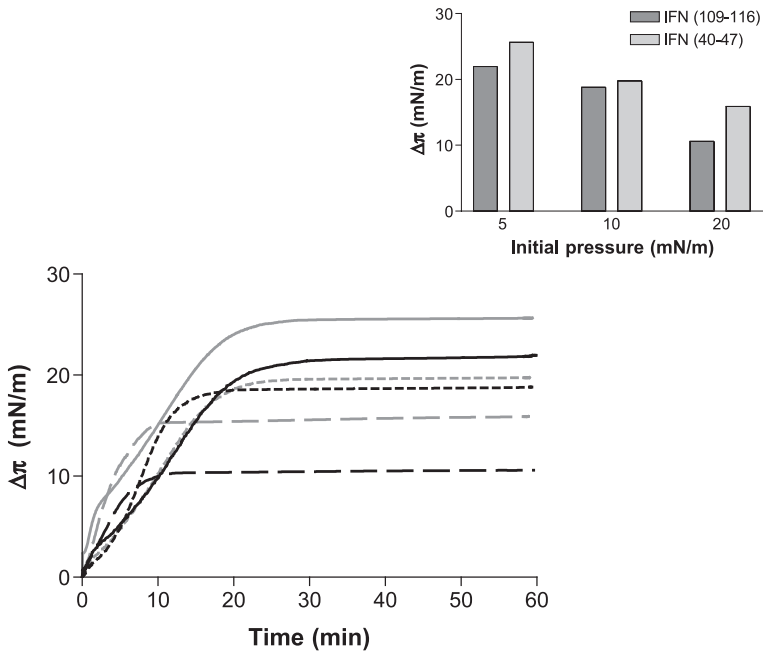

Fig. 2. Pressure increase produced by Palm-β-IFN(40–47) at (—) 5 mN/m, (- - -) 10 mN/m, (— —) 20 mN/m and Palm-β-IFN(109–116) at (—) 5 mN/m, (- - -) 10 mN/m, (— —) 20 mNm. Peptide concentration was 0.6 μM. The inset reports the pressure increase (mN/m) obtained after 1 h for Palm-β-IFN(40–47) and Palm-β-IFN(109–116) injected under DPPC membrane at different initial pressures.

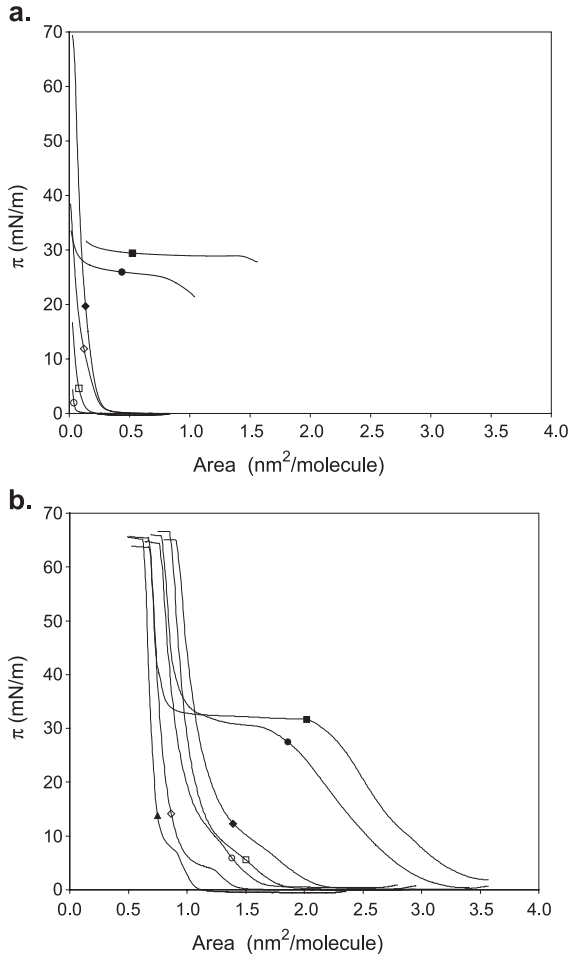

Fig. 3. (a) Compression isotherms of ◇ β-IFN(13–20), □ β-IFN(40–47), ○ β-IFN(109–116), ◆ Palm-β-IFN(13–20), ■ Palm-β-IFN(40–47), and ● Palm-β-IFN(109–116). (b) Compression isotherms of ▲ DPPC and DPPC spread on subphases containing the peptides at 1.8 μM.

hydrophobic molecules [14]. The contribution of the Palm-β-IFN(40–47) to the surface pressure increase is greater than the obtained for Palm-β-IFN(109–116), thus agreeing with the greater surface activity of Palm-β-IFN(40–47).

The interaction with DPPC could be related with the different hydrophobicity of the molecules studied. Accordingly, Palm-β-IFN(40–47) has a higher aliphatic index, having a value of 146.12. This index is defined as the relative volume of a peptide or a protein occupied by the aliphatic side chains (alanine, valine, isoleucine, and leucine) [15]. On the other hand, Palm-β-IFN(109–116) has a value of 61.25. Moreover, the Grand Average of Hydropathicity (GRAVY) parameter, calculated as the sum of hydropathy values of all the amino acids, divided by the number of residues in the sequence [16], was also higher for Palm-β-IFN(40–47) than for Palm-β-IFN(109–116).

3.4. Compression isotherms

In order to have a more complete knowledge of the physicochemical properties of β-IFN peptides, compression isotherms were studied. Fig. 3a shows the obtained isotherms of the different peptides. All free peptides showed very low area/molecule values that suggested a solubilization of the peptides into the subphase after being spread on the interface, this fact being in agreement with their lack of surface activity. As in previous experiments of surface activity and penetration kinetics, Palm-β-IFN(13–20) had a similar behaviour to free peptides. On the contrary, Palm-IFN(40–47) and Palm-IFN(109–116) formed a stable monolayer at the air/water interface. The peptide concentration used was 1.8 μM for all peptides, the hydrophobic peptides Palm-β-IFN(40–47) and Palm-β-IFN(109–116) presented elevated initial pressures but without all the ordered states being presented. Since the monolayers in these conditions were totally collapsed the isotherms of Palm-β-IFN(40–47) and Palm-β-IFN(109–116) were repeated with a lower concentration, 0.6 μM. Fig. 3b shows the obtained isotherms of surface pressure versus mean area for the pure lipid DPPC and DPPC with peptides in the subphase. Fixed amounts of DPPC (50 μl solution of 1 mg/ml in $CHCl_3$/MeOH 2/1 v/v, aprox. 4.10^{16} lipid molecules) were spread on the aqueous subphase containing the peptides under study at 1.8 μM.

The isotherm taken from pure DPPC shows the well-known phase transition from liquid-expanded (LE) to liquid-condensed (LC) phases around 7 mN m^{-1} and 0.9 nm^2 $molecule^{-1}$ of molecular area. DPPC monolayers spread onto the surface of peptide solution were shifted to higher areas compared to the isotherm obtained in the absence of peptide. All free peptides and Palm-β-IFN(13–20) did not change the ordered states; however, Palm-β-IFN(40–47) and Palm-β-IFN(109–116) showed higher area values at

Table 2
Surface compression modulus (mN/m) of monolayers of DPPC and DPPC with peptides in the subphase PBS

π (mN/m)	DPPC	DPPC β-IFN(13–20)	DPPC β-IFN(40–47)	DPPC β-IFN(109–116)	DPPC Palm-β-IFN(13–20)	DPPC Palm-β-IFN(40–47)	DPPC Palm-β-IFN(109–116)
5	26.55	8.24	22.09	25.24	25.19	38.06	37.44
10	17.64	31.85	20.37	20.35	18.36	44.53	52.84
20	76.23	61.59	59.52	44.50	46.06	77.11	52.15
30	113.69	94.51	94.64	77.36	85.54	26.94	9.35
40	132.67	95.87	135.31	101.74	106.34	27.54	39.23
50	155.27	141.98	167.67	133.55	151.92	121.87	128.83
60	75.56	81.15	107.58	82.52	101.90	79.18	84.05

pressures smaller than 30 mN/m. On the other hand, Palm-β-IFN(40–47) and Palm-β-IFN(109–116) presented area values smaller than the other peptides at higher pressures, suggesting that these peptides were released from the monolayer [17].

Surface compression modulus (C_s^{-1}) values of monolayers in the presence of DPPC were calculated using the values of Fig. 3b and applying Eq. (1) [18]:

$$C_s^{-1} = -A\left(\frac{\partial \pi}{\partial A}\right)_T \quad (1)$$

Results obtained (Table 2) show that in DPPC monolayers, Palm-β-IFN(40–47) and Palm-β-IFN(109–116) at lower pressures have a higher compressibility than free peptides and Palm-β-IFN(13–20). The maximum surface compression modulus was produced around 50 mN/m for all peptides studied.

The expanding effect caused by the peptides reflect a destabilization of the monolayer packing. The greater effect of the lipopeptides can be attributed to higher hydrophobic interactions as a consequence of its palmitoylated tail. Moreover, the different behaviour between the derivative peptides can be attributed to the different isoelectric point. Palm-β-IFN(40–47) and Palm-β-IFN(109–116) have isoelectric points of 4.5 and 4.4, respectively. Consequently, at pH 7.4 they were negatively charged. On the other hand,

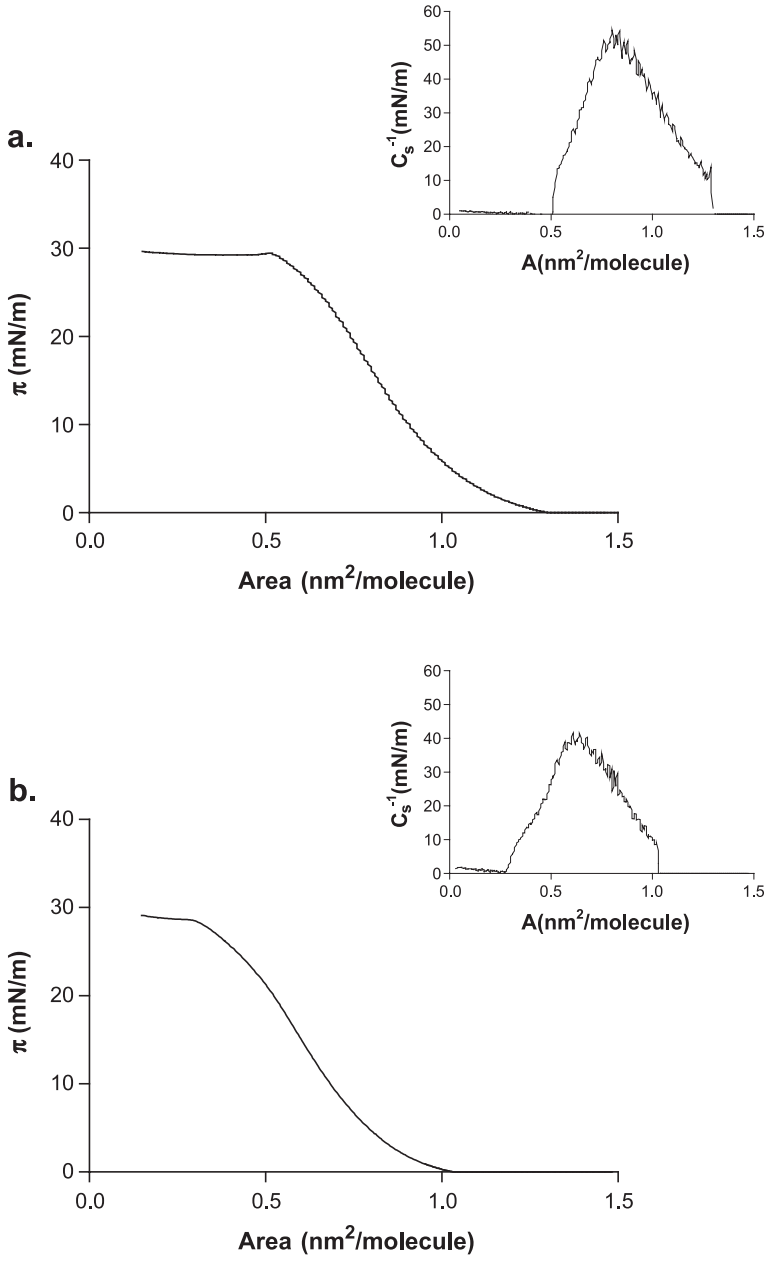

Fig. 4. Compression isotherms of (a) Palm-IFN(40–47) and (b) Palm-IFN(109–116) at 0.6 μM. Inset: surface compression modulus (C_s^{-1}) values as a function of the area/molecule.

Palm-β-IFN(13–20) has an isoelectric point of 7.9; thus, at this pH it would be neutral or positively charged.

The compression isotherms of Palm-IFN(40–47) and Palm-IFN(109–116) spreading 3.5×10^{17} molecules (0.6 μM) is shown in Fig. 4a and b. Palm-β-IFN(40–47) showed an extrapolated area/molecule of 1.05 nm^2/molecule and a lift-off area of 1.3 nm^2/molecule. The inset of the figure represents the compression modulus (C_s^{-1}) (mN/m) versus area (nm^2/molecule). Maximum C_s^{-1} values are around 50 mN/m, which suggests a liquid-expanded character. The Fig. 4b shows the isotherm of Palm-IFN(109–116) with an extrapolated area of 0.85 nm^2/molecule and a lift-off area of 1 nm^2/molecule. The compression modulus was similar to Palm-IFN(40–47) and there was the collapse without con-

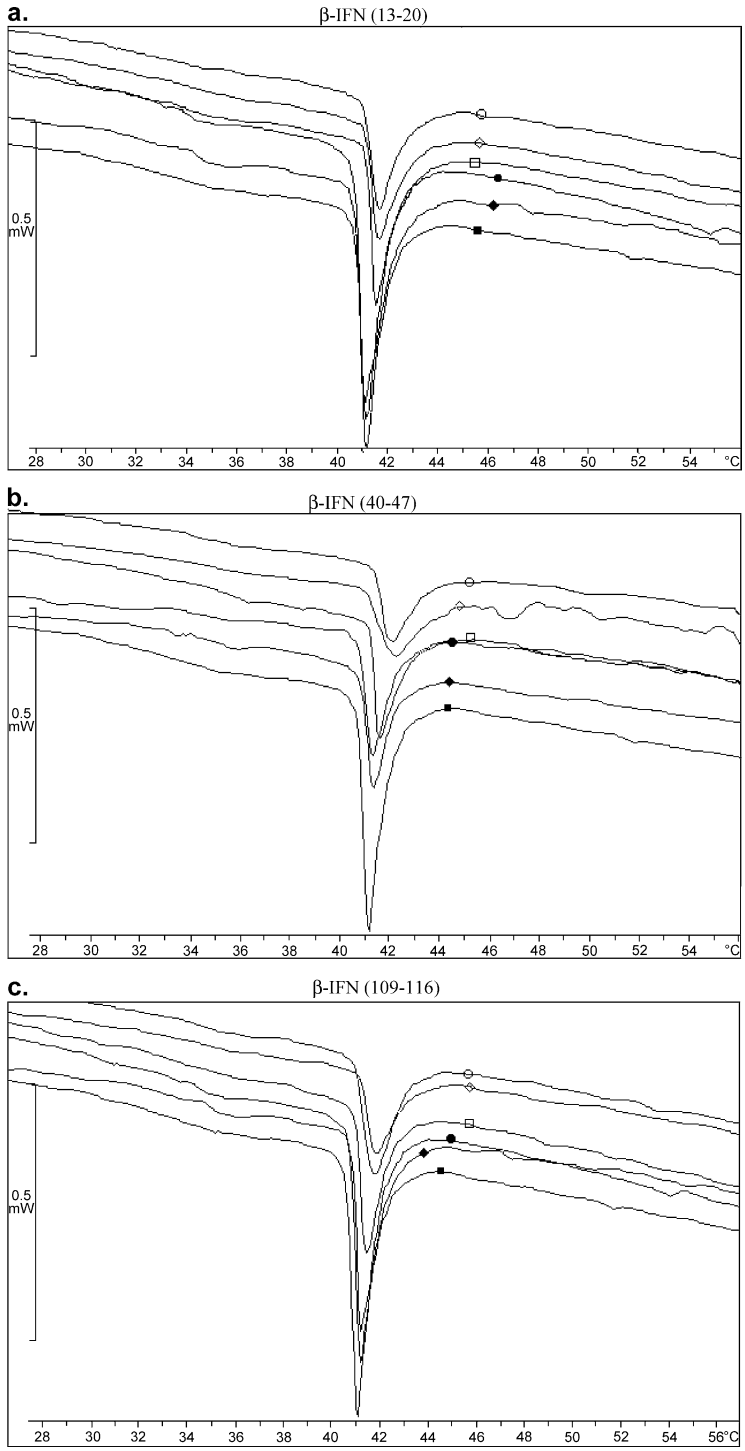

Fig. 5. DSC heating endotherms of DPPC MLVs were obtained in presence of 0 (■), 3 (♦), 5 (●), 10 (□), 20 (◇) and 30 (○) mol% of (a) β-IFN(13–20), (b) β-IFN(40–47) and (c) β-IFN(109–116). The curves refer to the second scan in the heating mode at a temperature scanning rate of 5 °C/min.

densed nor solid state. These values can provide different conformational states [19].

3.5. Differential scanning calorimetry (DSC)

The effect of β-IFN peptides on the gel–liquid crystalline phase transition of DPPC liposomal bilayer membranes was examined by differential scanning calorimetry (DSC). When the transition occurs upon increasing the temperature, several structural changes in the lipid molecules are produced. The principal change associated to the transition is the trans-gauche isomerization in the acyl chains and the average of gauche conformers is related to bilayer fluidity [20]. In Fig. 5 are presented the thermograms of MLV-DPPC alone and in the presence of increasing concentrations of the peptides. The multilamellar bilayers of DPPC alone showed a pretransition at 34.5 °C that is attributed to the transition of two different gel phases: Lβ phase to the rippled gel Pβ phase and a main phase transition (T_m) at 41 °C due to the chain melting transition Pβ to a highly cooperative transition to a lamellar liquid crystalline, Lα. The chain melting transition observed was sharp, the half width at the scan rate used (5 °C/min) being 0.7 °C, similarly to the values described in the literature [21]. The obtained total enthalpy change (36.05 kJ/mol) also agreed with the literature data [22]. When studied the influence of β-IFN peptides, we could observe that they caused a low perturbation in DPPC bilayers. The temperature of the main gel to liquid crystalline phase transition was practically not affected. The little change in the T_m for all peptides could suggest that the interactions of the studied peptides with DPPC vesicles did not alter the packing of the hydrocarbon chains in the gel and liquid crystalline states [23,24]. However, the peak becomes broader suggesting a lower cooperatively on the phospholipid main phase transition in presence of the β-IFN peptides [25]. As described by Ali et al. [26], an enthalpy decrease associated with increasing amounts of peptides (Table 3) could be attributed to a reduction of the intermolecular interactions between the hydrophobic region of the bilayer interiors, caused by the disruption of hydrogen bonding at the lipid/water interface produced by the peptides. In the palmitoylated peptides, the effect on the thermotropic parameters of DPPC MLVs was greater (Fig. 6). Similarly, as occurred with the monolayer technique, Palm-β-IFN(13–20) showed a lower interaction with MLV-DPPC compared to the other palmitoylated peptides. The decrease of enthalpy was about two times lower than the obtained for the other peptides and the peak did not disappear at the higher concentration of the peptide. However, Palm-IFN(40–47) and Palm-IFN(109–116) caused the total disappearance of the transition peak at a percentage of 20% of peptide. As in the other studied techniques Palm-β-IFN(40–47) interacts with a higher extent than Palm-β-IFN(109–116). In Fig. 7, the variation of the transition enthalpy versus the percentage of both free and lipophilically derivatised β-IFN peptides is shown.

Table 3
Thermotropic parameters of the gel to liquid crystalline phase transition of DPPC MLVs prepared in presence of the different peptides

DPPC	T_m (°C)	ΔH (kJ/mol)	$\Delta T_{1/2}$ (°C)
DPPC	41.5	36.0	0.8
β-IFN(13–20)			
3%	41.4	30.8	0.9
5%	41.8	32.5	1.1
10%	41.6	28.4	0.9
20%	41.6	23.3	1.1
30%	41.6	22.0	1.1
β-IFN(40–47)			
3%	41.4	20.8	0.9
5%	41.3	21.1	0.9
10%	41.5	21.7	1.0
20%	42.2	17.5	1.8
30%	42.1	17.5	1.4
β-IFN(109–116)			
3%	41.4	30.6	0.9
5%	41.9	32.7	1.1
10%	41.3	28.9	0.9
20%	41.8	18.3	1.3
30%	41.8	18.1	1.4
Palm-β-IFN(13–20)			
3%	41.2	29.3	1.1
5%	41.1	27.6	1.2
10%	42.2	34.7	1.3
20%	41.7	24.5	2.3
30%	41.2	21.0	1.8
Palm-β-IFN(40–47)			
3%	41.5	29.2	1.5
5%	42.5	24.0	3.7
10%	42.5	8.1	2.7
20%	–	–	–
30%	–	–	–
Palm-β-IFN(109–116)			
3%	41.3	24.9	1.1
5%	41.2	30.6	1.6
10%	41.5	8.4	3.4
20%	–	–	–
30%	–	–	–

To sum up, the studied peptides did not cause any significant change in the transition temperature; nevertheless, they produced a clear decrease in the enthalpy and a broadening of the transition peak. This behaviour is characteristic of molecules with both hydrophobic and hydrophilic interactions with phospholipid membranes being thus mainly situated in the outer part of the bilayer but also having a partial interfacial location [27].

In order to get more insights into the main forces that contribute to peptide–lipid interactions, we tried to evaluate and to compare whether different electrostatic interactions were established between the two negatively charged synthetic lipopeptides [Palm-β-IFN(40–47) and Palm-β-

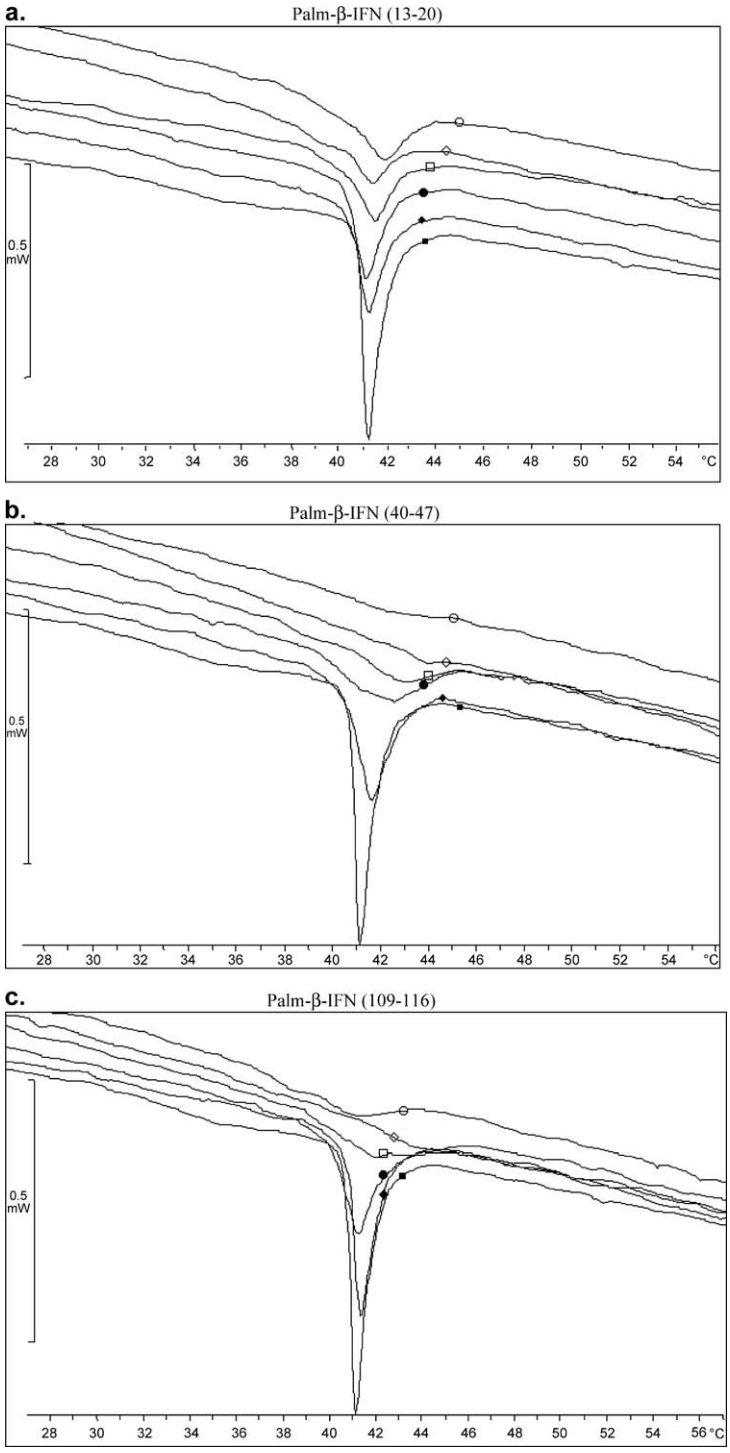

Fig. 6. DSC heating endotherms of DPPC MLVs were obtained in presence of 0 (■), 3 (♦), 5 (●), 10 (□), 20 (◇) and 30 (○) mol% of (a) Palm-β-IFN(13–20), (b) Palm-β-IFN(40–47) and (c) Palm-β-IFN(109–116).

IFN(109–116)] and the positively charged one, Palm-β-IFN(13–20), with a negative phospholipid, such as dipalmitoylphosphatydilglicerol (DPPG). As described in the literature [28], the DSC curve of MLV-DPPG showed an endothermic peak at 40.7 °C due to the main phase transition temperature. Table 4 lists quantitative data from the thermograms studied, in the absence and in the presence of 10% of palmitoylated peptides. MLV-DPPG experimented a light increase in the transition temperature when the peptides were added. Moreover, in all cases, the broadening of the transition profile of DPPG bilayers was considerable. Regarding ΔH values, Palm-β-IFN(13–20) produces a light increase. This result could be explained by a certain rigidification of the bilayer, mainly produced by

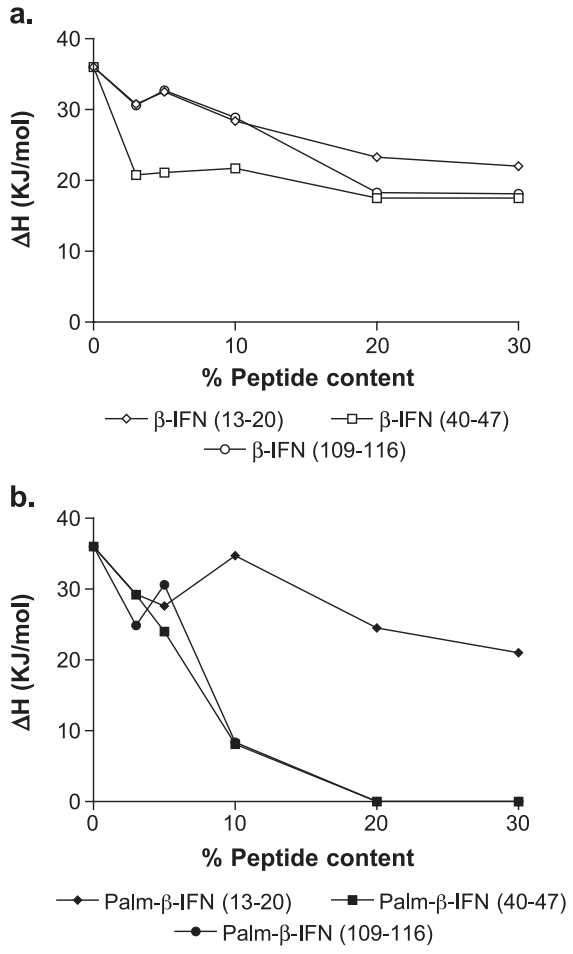

Fig. 7. Dependence of the transition enthalpy (ΔH) of the main gel to liquid crystalline phase transition of DPPC on the percentage of peptides (a) β-IFN peptides and (b) Palm-β-IFN peptides.

electrostatic forces between the positively charged peptide, that could be located at the outer part of the bilayer without penetrating into the bilayer interior, and the negative character of MLVs-DPPG.

On the other hand, the two negatively charged peptides, this is Palm-β-IFN(40–47) and Palm-β-IFN(109–116), produced a notorious effect on ΔH, these values being clearly lower. These results agree with a decrease in acyl chain cooperativity of the bilayer possibly due to a partial penetration of the lipidic chain and the consequent deformation of the packing of the phospholipid acyl chains. In these cases, the interaction peptide/lipid is produced mainly by hydrophobic forces.

4. Conclusions

The present study was undertaken in order to investigate the interaction of peptides belonging to β-IFN with lipid model membranes (monolayers and liposomes). We have used different physicochemical techniques to analyse the properties of peptides and their palmitoylated sequences. The results obtained suggest the following conclusions:

1. β-IFN(13–20), β-IFN(40–47) and β-IFN(109–116) do not have surface activity due to the elevated solubility in the aqueous subphase. Palm-β-IFN(13–20) although bearing a palmitoyl hydrophobic tail, it behaves like a hydrophilic peptide without being detected surface activity. Pressure increase of DPPC monolayers in the presence of Palm-β-IFN(40–47) and Palm-β-IFN(109–116) was studied because they have shown enough surface activity. Palm-β-IFN(40–47) shows a greater effect than Palm-β-IFN(109–116) in all initial pressures studied. Having in mind that the fatty acid that was incorporated in free β-IFN peptides was the same, the different effects observed in the DPPC monolayer could be attributed to the different amino acid sequence within the two peptides and the different conformation adopted.
2. β-IFN(13–20), β-IFN(40–47), β-IFN(109–116) and Palm-IFN(13–20) do not form stable monolayers when spread at an air/water interface due to their high hydrophilicity. However, Palm-β-IFN(40–47) and Palm-β-IFN(109–116) do form stable monolayers, but not showing all the ordered states at the same studied concentrations than the free peptides. On the other hand, at a lower concentration Palm-β-IFN(40–47) and Palm-β-IFN(109–116) presented all the ordered states. The different effect of peptides in monolayers could be attributed to the different isoelectric point.
3. The effects of the free peptides on the thermotropic phase transition properties of MLV DPPC have shown that β-IFN(40–47) shows the greater effect. Although the peptides do not show a significant displacement of the DPPC phase transition midpoint, at 20% β-IFN(40–47) a small shift to lower temperatures was observed. ΔH clearly decreases with an increase in the peptide content, being the decrease observed of 39%, 51% and 50% for β-IFN(13–20), β-IFN(40–47) and β-IFN(109–116), respectively. Moreover, the main transition peak broadens with increasing the amount of peptide present. The change on the thermotropic parameters of the DPPC in the presence of palmitoylated peptides is similar than the one obtained for the free peptides at low concentrations. The phase transition of DPPC disappears at high peptide/

Table 4
Thermotropic parameters of the gel to liquid crystalline phase transition of MLVs DPPG prepared in presence of palmitoylated β-IFN peptides

	T_m (°C)	ΔH (kJ/mol)	$\Delta T_{1/2}$ (°C)
DPPG	40.7	27.0	1.3
DPPG/Palm-β-IFN(13–20)	40.8	29.1	2.4
DPPG/Palm-β-IFN(40–47)	41.6	18.9	3.8
DPPG/Palm-β-IFN(109–116)	41.6	18.1	3.3

The percentage of peptides was 10%.

phospholipid ratios in Palm-β-IFN(40–47) and Palm-β-IFN(109–116); however, at 30% content of Palm-β-IFN(13–20) the transition from the liquid crystalline to the gel phase could still be detected.

4. To evaluate the electrostatic interactions between phospholipids and the studied peptides, an anionic phospholipid such as DPPG, was chosen. As shown, Palm-β-IFN(40–47) and Palm-β-IFN(109–116) could penetrate deeper in the bilayer of MLVs having as a consequence a fluidification of the vesicles. On the other hand, Palm-β-IFN(13–20) could be preferentially located in the outer part of the bilayer.

This paper focused mainly on the physicochemical properties of synthetic β-IFN related peptides and their interaction with lipid model membranes. Future work will be carried out on the use of the described peptides as immunoreagents trying to correlate the properties herein described with their antigenic traits.

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