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The interaction of a peptide with a scrambled hydrophobic/hydrophilic sequence (Pro-Asp-Ala-Asp-Ala-His-Ala-His-Ala-His-Ala-Ala-Ala-His-Gly) (PADH) with DPPC model membranes: a DSC study

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Abstract

Depending on their hydrophobicity, peptides can interact differently with lipid membranes inducing dramatic modifications into their host systems. In the present paper, the interaction of a synthetic peptide with a scrambled hydrophobic/hydrophilic sequence (Pro-Asp-Ala-Asp-Ala-His-Ala-His-Ala-His-Ala-Ala-Ala-His-Gly) (PADH) with 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) model membranes has been investigated by differential scanning calorimetry (DSC), adopting three different experimental approaches. In the first, the peptide is forced to be included into the hydrocarbon region of the lipid bilayer, by codissolving it with the lipid giving rise to mixed multilamellar vesicles–peptide systems; in the second, this system is passed through an extruder, thus producing large unilamellar vesicles–peptide systems; in the third, it is allowed to interact with the external surface of the membrane.

The whole of the DSC results obtained have shown that the incorporation of the peptide into the lipid bilayer by means of the first method induces a decrease in the enthalpy of the gel–liquid crystal transition of the membrane and a shift of the transition to the lower temperatures, thus resembling, in spite of its prevalently hydrophilic nature, the behavior of transbilayer hydrophobic peptides. The extrusion of these systems creates unilamellar vesicles free of peptides but of smaller size as evidenced by the decreased cooperativity of the transition. The peptide, added externally to the DPPC model membrane, has no effect on the phase behavior of the bilayer.

These findings suggest that the effect of the interaction of scrambled hydrophobic/hydrophilic peptides into lipid bilayers strongly affects the thermotropic behavior of the host membrane depending on the preparation method of the lipid/peptide systems. The whole of the results obtained in the present paper can be useful in approaching studies of bioactive peptides/lipids systems. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: DPPC; Lipid membrane; Differential scanning calorimetry; Interaction

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1. Introduction

The lipid component of prokaryotic and eukaryotic cell membranes is formed by a complex mixture of phospholipids, giving rise to different phases [1,2], depending on the different individual lipid classes present in such membranes, pH and ionic strength. A considerable body of evidence has now accumulated indicating that the various phase-preferring lipid components play important structural and functional roles in eukaryotic membranes [2]. The phase that a fully hydrated membrane lipid prefers under a given set of conditions can be rationalized by considering the geometric packing [3] and, on turn, the curvature stress induced in the bilayer [4]. The modulation of both these two factors by inclusions, such as proteins and peptides, can markedly affect the phase behavior of lipid membranes [5]. The α-helical conformation and transbilayer orientation of synthetic peptides [6–11] within lipid bilayers have been proven by a combination of different spectroscopic and X-ray diffraction measurements [12]. DSC [13] and ^{2}H NMR spectroscopic studies [14] have shown that the incorporation of the peptides into 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) bilayers and DPPE bilayers broadens the gel–liquid crystalline phase transitions and reduces its enthalpy. The effect of the hydrophobic length of the peptide is usually invoked to explain the promotion of the preferred phase [9].

One of the common structural features in biologically active peptides and proteins, such as polypeptide hormones, polypeptide antibiotics and venoms is an α-helical structure in which the aminoacidic sequence has both hydrophobic and hydrophilic character [15]. The arrangement of hydrophobic/hydrophilic residues in the sequence has been shown to modulate the cell-lytic properties of the α-helical transbilayer peptide [16–18]. For these reasons, studies on peptides/membrane association phenomena have been recently concentrated on the modifications of the physical parameters of the lipidic bilayer as a consequence of peptide incorporation. However, in spite of their potential importance, the effects of different methods of peptide/membrane incorporation on the thermotropic behaviour of the bilayer, are poorly investigated.

In this light, here we report the DSC measurements of a DPPC/peptide system where the peptide chosen as a model was a fragment (Pro-Asp-Ala-Asp-Ala-His-Ala-His-Ala-His-Ala-Ala-Ala-His-Gly) (PADH) with a scrambled hydrophobic/hydrophilic sequence. This peptide has been shown to possess a considerable propensity to form stable α-helices and therefore is a good candidate for this kind of studies [19]. The DSC results have been discussed in terms of different preparation protocols of the lipid/peptide systems, thus pointing out the not negligible effect of the sample preparation in avoiding artifacts in the interpretation of results.

2. Experimental

2.1. Chemicals

The peptide (Pro-Asp-Ala-Asp-Ala-His-Ala-His-Ala-His-Ala-Ala-Ala-His-Gly) (PADH) was synthesized on a Milligen 9050 peptide synthesizer as previously reported [19].

DPPC was obtained from FLUKA.

All inorganic salts for phosphate buffer preparation were purchased from Sigma Chemical co.

2.2. Preparation of pure DPPC multilamellar vesicles (MLV)

Pure membranes were prepared drying DPPC/$CHCl_3$ solutions by evaporating them under high vacuum to dryness in round-bottomed flasks and by removing all residual solvent by a gentle nitrogen flow. The resulting lipid film on the wall of the flask was then hydrated with an appropriate volume of 10 mM phosphate buffer (pH 7.0 and 0.1 M ionic strength in NaCl) and dispersed by vigorous stirring in a water bath set at 50–55 °C. The final nominal concentration of the lipid was 2 mg/ml.

2.3. Preparation of pure DPPC large unilamellar vesicles (LUV)

The multilamellar vesicles were extruded through polycarbonate filters (pore size = 100 nm) (Nuclepore, Pleasanton, CA) mounted in a mini-extruder (Avestin Inc.) fitted with two 0.5 ml Hamilton gastight syringes (Hamilton, Reno, NV). Usually we subjected samples to 19 passes through two filters in tandem

as recommended elsewhere [20]. An odd number of passages were performed to avoid contamination of the sample by vesicles that might not have passed through the filter.

2.4. Incorporation of peptide fragments in model membranes

Three different protocols have been applied to prepare mixed lipid/peptide bilayers:

(A) The peptide fragment was dissolved in the same organic solution ($CHCl_3$) of the phospholipid (molar ratio lipid/peptide $x = 0.1$). The peptide/lipid organic solution was dried under nitrogen and evaporated under high vacuum to dryness in round-bottomed flasks. The MLV membrane was then obtained as above described.

(B) The DPPC MLV/peptide model membrane were extruded as previously described to prepare large unilamellar vesicles of DPPC/peptide (DPPC LUV/peptide).

(C) A proper amount of peptide was added to previously prepared pure DPPC LUVs membrane suspensions to give a final lipid/peptide molar ratio of 10/1 ($x = 0.1$). The mixture was initially vigorously vortexed for 1–2 min, and if not otherwise specified, immediately scanned.

A drawing describing the three different preparation protocols is reported in Fig. 1.

2.5. Differential scanning calorimetry

DSC scans were carried out with a second generation high-sensitivity SETARAM micro differential scanning calorimeter (microDSC II) with 1 ml stainless steel sample cells, interfaced with a BULL 200 Micral computer. The sampling rate was 1 point/s in all measuring ranges. The same solution without the sample was used in the reference cell. Both the sample and reference were heated with a precision of 0.05 °C at a scanning rate of 0.5 K/min. In order to obtain the excess heat capacity ($C_{p_{exc}}$) curves, buffer–buffer base

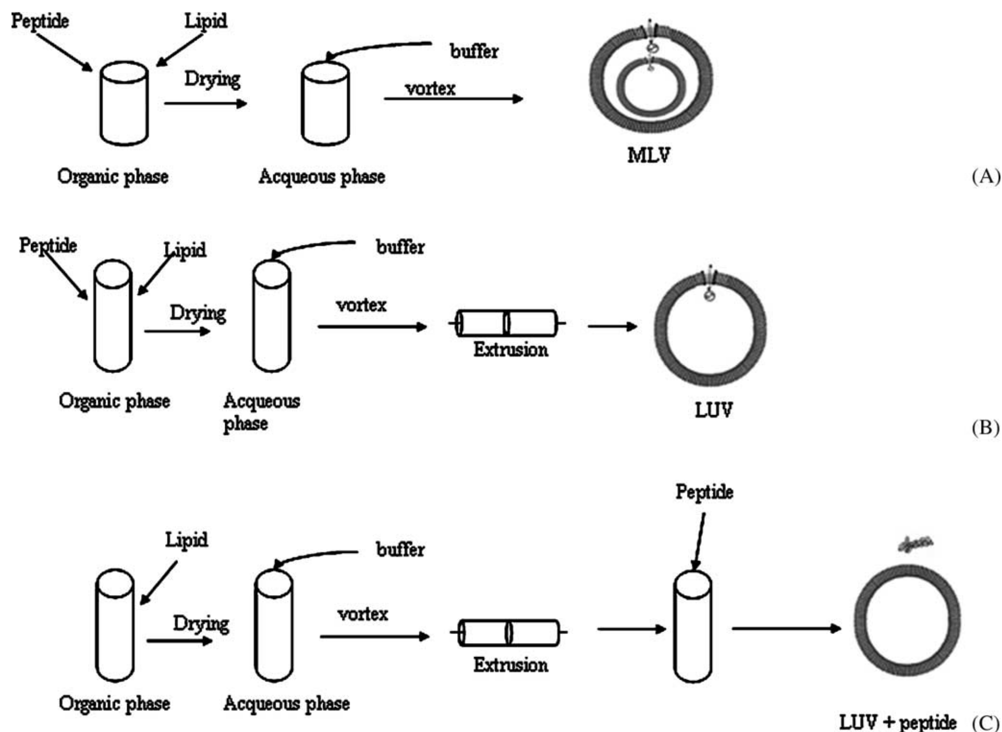

Fig. 1. Schematic drawing of the three possible preparation methods of the lipid/peptide systems reported in Section 2.

lines were recorded at the same scanning rate and then subtracted from sample. The average level of noise was about ± 0.4 μW and the reproducibility at refilling was about 0.1 mJ/K/ml.

Calibration in energy was obtained by giving a definite power supply, electrically generated by an EJ2 SETARAM Joule calibrator within the sample cell. To check the reproducibility of the results, three different samples were scanned. For each sample, three heating and two cooling scans were recorded. Cooling scans yielded curves very similar to the heating scans, but the transitions in cooling curves are shifted, by about 1 °C, to lower temperatures. Therefore, due to the supercooling phenomenon, accurate thermotropic transitions are evaluated from heating curves. For this reason, only heating scans have been discussed in the present work. In the present work only the main transition of DPPC model membranes is considered because the pre-transition is strongly dependent on the preparation method of the membrane and it disappears when the liposomes are extruded [4].

3. Results and discussion

In the upper panel of Fig. 2, the $C_{p_{exc}}$ profile of pure (curve a) MLVs-DPPC and peptide/MLVs-DPPC (curve b) bilayer, prepared according to method A described in the experimental section, are reported. The corresponding thermodynamic parameters are reported in Table 1. It can be noted that the DSC peak relating to the peptide/DPPC system is broadened and shifted to lower temperatures with respect to the pure lipid curve. In particular, the T_m is lowered by about 1.5 °C and the enthalpy is decreased to 28.3 kJ/mol, with respect to the pure DPPC (see Table 1). Moreover the cooperativity of the transition decreases as evidenced by an increase of the width of the DSC peak at half height (W) from 1.51 to 2.19 °C. This behavior, which has been previously observed for other trans-bilayer hydrophobic peptides [21] can be explained if we consider that the peptide acts as a "defect" into the membrane bilayer because of its random dispersion in the hydrocarbon region. In this case the electrostatic charges and the geometric features of the guest molecule perturb the lipid–lipid interactions, giving rise to a decrease in the rigidity and in the stability of the membrane. The DSC peak relative to such a peptide/membrane system, if compared with the behaviour of a pure membrane, should exhibit lower T_m, ΔH and sharpness which is believed to be related with the rigidity of the membrane.

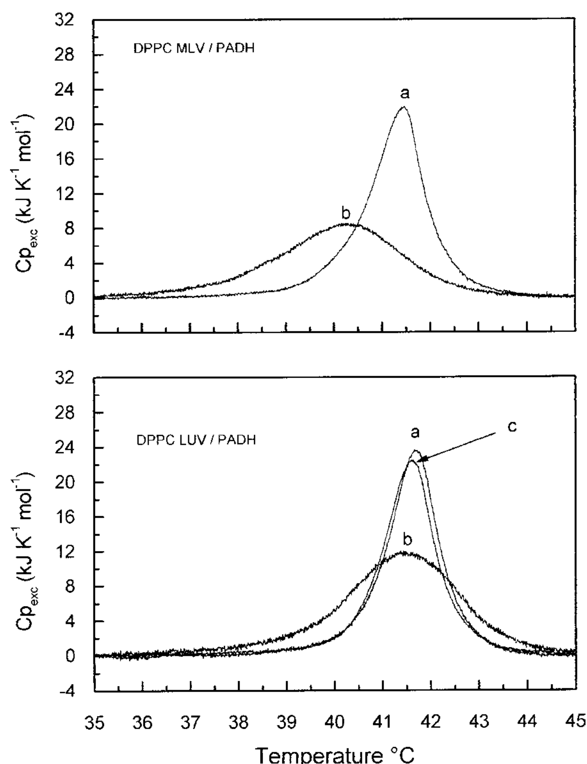

Fig. 2. Upper Panel DSC curves for pure DPPC MLV membranes (curve a) and mixtures of peptide/DPPC (peptide/lipid molar ratio 1:10) MLV prepared according to method A (curve b). Lower Panel DSC curves for mixtures of peptide/DPPC (peptide/lipid molar ratio 1:10) Large Unilamellar Vesicles prepared according to methods B (curve b) and C (curve c). In the same panel curve (a) represents the DSC transition of a pure DPPC LUV membrane. The heating rate was 0.5 K/min. All the samples were prepared in phosphate buffer 10 mM pH = 7.0, ionic strength was adjusted to 0.1 M by sodium chloride.

In order to gain insights into the peptide/cell membrane association phenomenon, the thermotropic behaviour of large unilamellar vesicles (LUVs) of lipid/peptide systems has been also investigated. LUVs produced by extrusion techniques as reported in the experimental section, present several advantages: they are free of organic solvent and detergent; they are relatively homogeneous in size and structure; they resemble cell membranes in being unilamellar and able to contain relatively large volumes.

Table 1
Calorimetric peak temperatures (T_m), total enthalpy changes (ΔH) and width of the peak at half height (W) relative to the different peptide/lipid bilayer systems

		T_m (°C)	ΔH (kJ/mol)	W (°C)
DPPC MLV	Fig. 2 upper panel curve a	41.7 ± 0.05	38.3 ± 0.6	1.51 ± 0.08
DPPC LUV	Fig. 2 lower panel curve a	41.6 ± 0.06	36.0 ± 0.9	1.19 ± 0.02
DPPC MLV/PADH	Fig. 2 upper panel curve b	40.3 ± 0.07	28.3 ± 0.8	2.17 ± 0.05
DPPC LUV/PADH	Fig. 2 lower panel curve b	41.4 ± 0.04	36.0 ± 0.7	2.00 ± 0.06
DPPC LUVs ± PADH	Fig. 2 lower panel curve c	41.6 ± 0.05	35.6 ± 0.6	1.10 ± 0.04

The preparation of each different system and the experimental conditions adopted are described in Section 2. Experimental values are reported as mean ± S.D. of at least three repeated experiments.

In the lower panel of Fig. 2 the DSC curves of LUVs of pure DPPC (curve a), and peptide/DPPC (curve b) prepared according to method B described in the experimental section are reported. The corresponding thermodynamic parameters are reported in Table 1. It can be noted that as a consequence of the extrusion process, the obtained peptide/DPPC system shows, in spite of a broadened curve, a transition temperature (T_m), and enthalpy ΔH very similar to the pure DPPC system. In particular, T_m is lowered by only 0.2 °C and the enthalpy is decreased by about 2 kJ/mol, with respect to the pure DPPC (see Table 1). Moreover the cooperativity of the transition decreases as evidenced by an increase in W from 1.10 to 2.00 °C.

In order to test the interaction of the peptide with the external membrane surface a peptide/DPPC system was prepared according to method C and scanned by DSC (Fig. 2, lower panel, curve c). It is evident that when the peptide is added to the aqueous suspension after the formation of the vesicles, no interaction with the lipid bilayer is detected. The DSC curve of the same system incubated for 2 h at 4 °C above the transition state does not change. This result confirms that the peptide does not interact with membrane when the membrane is in the liquid crystal phase, at which the incorporation of peptides into membrane is supposed to be maximal [22].

Despite the partial hydrophilic nature of the PADH peptide, the calorimetric behavior observed for the lipid/peptide system prepared according to method A and reported in the upper panel of Fig. 2 closely resembles the behavior of an α-helical transmembrane peptide with a predominantly hydrophobic sequence [21]. On the contrary, when the peptide is allowed to interact with the external surface of the bilayer by adopting the preparation protocol C described in the experimental section, no effects on the thermotropic behavior of the bilayer has been evidenced. This means that when the lipid and the peptide are codissolved together in the organic phase during the preparation of the membrane, the peptide is somehow "entrapped" into the bilayer causing a broadening of the DSC curve and a decrease of the enthalpy associated with the gel/liquid crystal transition of the lipid bilayer, thus mimicking the thermotropic behavior of a membrane perturbed by a hydrophobic transmembrane peptide. On the other hand, when the peptide is added to the membrane suspension after the formation of the bilayer, the scrambled hydrophobic/hydrophilic nature of the peptide is not sufficient to induce evident lipid–peptide interactions. An even more intriguing aspect has been evidenced by the investigation of the thermotropic behavior of a lipid/peptide system prepared according to method B. In this case the temperature at which the main transition occurs and the observed enthalpy are similar to the ones detected for pure membranes, but the cooperativity of the transition is decreased. These observations are consistent with the hypothesis that when a multilamellar lipid/peptide system prepared according to method A, is extruded through proper filters the peptide could partially leave the bilayer going into aqueous solution inducing a broader distribution of the vescicle size [4].

4. Conclusions

The primary structure has been shown to play an important role in cell-lytic and antimicrobial peptides that act by perturbing the barrier function of membranes [16–18,23]. Depending on their hydrophobic/

hydrophilic balance, the peptides either stabilize or lyse the membrane [17,24]

Some authors have recently analyzed the relationship between the relative magnitude of the hydrophobic–hydrophilic moiety and membrane-binding properties [25–27]. It turned out that the interaction of peptides with membranes mainly involves two binding properties: (l) hydrophobic interaction between the lipid acyl chain and the hydrophobic residues of the peptide; (2) electrostatic interactions among the polar residues of the peptides, the phospholipid headgroup and the solvent molecules.

However, for a correct interpretation of the effects that these factors have on the thermotropic behaviour of the membrane, it is important, as evidenced in the present paper, to take properly into account the choice of the sample preparation method. In fact, if the peptide and the lipids are mixed together before the assembling of the bilayer, the peptide is entrapped into the model membrane, thus inducing deep modifications in the properties of the bilayer. This does not necessarily means that the peptide is able to spontaneously interact with the cell membrane in acqeuous solution. In order to prove this, it is necessary to mix pre-formed unilamellar vesicles with the peptide and investigate the eventual consequent modifications of the bilayer properties.

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