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Perturbations induced by synthetic peptides belonging to the E2 structural protein of hepatitis G virus (GBV-C/HGV) in lipid membranes: A differential scanning calorimetry study

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Abstract: In the present work we report the perturbations induced on multilamellar liposomes (MLVs) of different phospholipid composition (DPPC, DMPC, DMPC/DMPG) by peptides that belong to hepatitis G virus (GBV-C/HGV). A differential scanning calorimetry (DSC) study was performed with MLVs in the presence of increasing amounts of the three synthetic overlapping peptides from GBV-C/HGV, namely E2(17-26), E2(12-26) and E2(7-26) sequences.

1. INTRODUCTION

Lipid membranes are characterised by a main phase transition between an ordered gel state and a disordered liquid-crystalline phase. The investigation of the thermotropic changes in liposome behaviour in presence of peptide molecules provides useful data on the nature, depth of their interactions and localization within the membrane model [1,2]. In the present work the thermotropic behaviour of dipalmitoyl phosphatidylcholine (DPPC), dimyristoyl phosphatidylcholine (DMPC) and dimyristoyl phosphatidylcholine / dimyristoyl phosphatidylglycerol (DMPC / DMPG) multillamelar vesicles was studied by differential scanning calorimetry (DSC), in the absence and in the presence of increasing concentrations of three overlapping E2 (GBV-C/HGV) belonging peptides. A comparison of the effect of the 10-, 15- and 20-mer peptides on the thermotropic parameters of the lipid vesicles is reported.

2. EXPERIMENTAL PART

2.1 Chemicals

DPPC, DMPC, and DMPG were from Avanti Polar Lipids. Chloroform and methanol were from Merck and Carlo Erba, respectively. Water was double distilled. The buffer used was 5 mM HEPES pH 7.4.

2.2 Peptide Synthesis

The synthesis and analytical characterization of peptides will be described elsewhere. Crude peptides were purified by semi preparative HPLC. Purified compounds were characterized by analytical HPLC, amino acid analysis and electrospray mass spectrometry.

2.3 Differential scanning calorimetry (DSC) measurements

DPPC, DMPC, DMPC/DMPG (2/1) were dissolved in a chloroform/methanol (2:1) mixture and the lipid solution was dried under nitrogen. The samples were stored in a vacuum oven overnight at room temperature to eliminate the residual solvent. The lipid films were hydrated with Hepes buffer (5mM, pH 7.4). MLVs were obtained by vortexing the mixture, always keeping the temperature above the highest gel to liquid-crystalline phase transition of the sample. Multilamellar vesicles containing 6.10^{-4} mmol of DPPC or 3.10^{-3} mmol of DMPC and DMPC/DMPG (2:1) were analysed by DSC alone or with peptides at different lipid/peptide molar ratios (100/0, 98/2, 95/5, 90/10, 80/20, 70/30).
30 µl of MLVs were sealed in small aluminium calorimetry pans and submitted to heating/cooling cycles. Scans were carried out in a DSC 821E Mettler Toledo calorimeter, at heating and cooling rates of 5° C min^{-1}over the sample from 0°C to 60°C. The calorimeter was calibrated with Indium.

3. RESULTS AND DISCUSSION

In Table I is shown the effect on the thermotropic parameters of MLVs of different lipid composition after the addition of 20% of the three studied peptides. The chain melting transition (Tm) of DPPC and DMPC was not significantly affected by the peptides. This observation suggests that the interactions of the peptides with these phospholipids do not alter significantly the packing of hydrocarbon chains in the gel and liquid crystalline states [3]. The melting profile for the 2:1 binary mixture of DMPC/DMPG vesicles shifted to higher temperatures for the three peptides showing a higher displacement working with the E2(7-26) peptide sequence. The enthalpy (ΔH) at 20% molar ratio decreased in all cases showing a higher effect when the peptide chain length increased.

Table I. Thermotropic Parameters of the gel to liquid crystalline phase transition of DPPC, DMPC and DMPC/DMPG (2:1) MLVs were prepared in presence of different peptides, E2 (17-26), E2 (12-26) and E2 (7-26), at a 20% mol.

	Tm[a] (°C)	ΔH (KJ/mol)	$\Delta T_{1/2}$[b] (°C)
DPPC	41.5	34.8	0.7
E2(17-26)	41.7	27.9	1.1
E2(12-26)	41.6	25.5	1.0
E2 (7-26)	41.3	25.2	0.7
DMPC	23.4	18.8	0.7
E2(17-26)	23.6	17.2	1.3
E2(12-26)	23.2	11.2	1.8
E2 (7-26)	23.7	10.9	2.0
DMPC/DMPG(2:1)	23.8	20.5	2.2
E2(17-26)	24.4	16.7	2.6
E2(12-26)	25.8	13.0	4.3
E2 (7-26)	27.6	9.4	5.9

[a]Main transition peak temperature. [b]Temperature width at half-height of the heat absorption peak.

The width ($\Delta T_{1/2}$) of the transitions did not change significantly in DPPC MLVs, however in presence of DMPC and DMPC/DMPG the transition broadened with the incorporation of the peptides, showing a greater effect the E2(7-26) peptide sequence.
The different behaviour between the three peptides could be attributed to the length of the chain as well as the different net charge of the peptides that would favour hydrophobic interactions [4]. E2(17-26) and E2(7-26) are positively charged peptides, while E2(12-26) is neutral, so as expected, electrostatic interactions between the positive peptides and the DMPG have probably been established. Nevertheless, we expected more interaction in positively peptides, but E2(12-26) interacted in a higher extent than did E2(17-26). For this reason, we can conclude that electrostatic interactions seem to play a minor role for the mixing of MLVs composed of DMPC/DMPG with the peptides.
As an example, in figure 1 the DSC profiles of mixtures of E2(7-26) with DMPC/DMPG MLVs are shown. As can be observed, the endothermic peak of the main transition decreased with the incorporation of the peptide and broadened significantly. At 30% peptide concentration the chain melting transition disappeared.

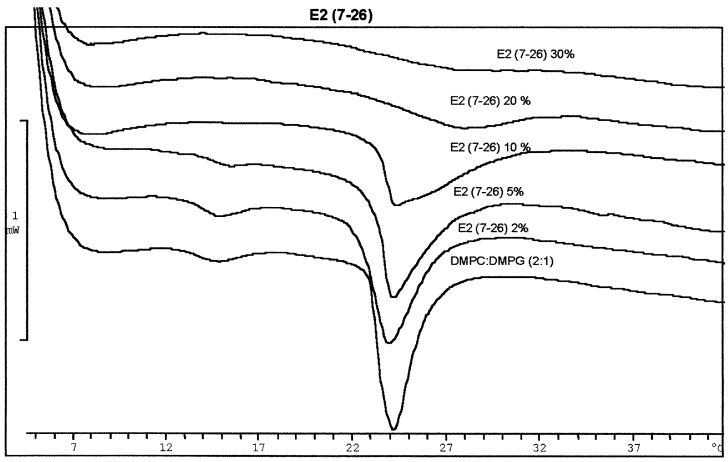

Figure1. DSC heating endotherms of DMPC/DMPG (2:1) MLVs were obtained in presence of 0, 2, 5,10, 20 and 30 mol % of E2 (7-26). The curves refer to the second scan in the heating mode at a temperature scanning rate of 5° C/min.

4. CONCLUSION

The main conclusion of this work is that the larger peptide chain the greater peptide-MLVs interaction measured by differential scanning calorimetry. Moreover, there is a stronger interaction with MLVs composed of DMPC/DMPG probably due to a combination of electrostatic and hydrophobic interactions. At high peptide/lipid ratios, the gel/liquid-crystalline phase transition disappears. This fact can be related with the perturbation and consequently the opening of the structure of MLVs caused by GBV-C/HGV peptides.

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