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Selective destabilization of acidic phospholipid bilayers performed by the hepatitis B virus fusion peptide

Ignacio Rodríguez-Crespo [a], Belén Yélamos [a], Juan P. Albar [b], Darrell L. Peterson [c], Francisco Gavilanes [a,*]

[a] *Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas, Universidad Complutense, 28040 Madrid, Spain*
[b] *Departamento de Inmunología y Oncología, Centro Nacional de Biotecnología, Unidad de Inmunología, CSIC-Pharmacia and Upjohn, Universidad Autónoma, 28049 Madrid, Spain*
[c] *Department of Biochemistry and Molecular Biophysics, Virginia Commonwealth University, Richmond, VA 23298, USA*

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Abstract

A peptide corresponding to the N-terminal region of the S protein of hepatitis B virus (Met-Glu-Asn-Ile-Thr-Ser-Gly-Phe-Leu-Gly-Pro-Leu-Leu-Val-Leu-Gln) has been previously demonstrated to perform aggregation and destabilization of acidic liposome bilayers and to adopt a highly stable β-sheet conformation in the presence of phospholipids. The changes in the lipid moiety produced by this peptide have been followed by fluorescence depolarization and electron microscopy. The later was employed to determine the size and shape of the peptide-vesicle complexes, showing the presence of highly aggregated and fused structures only when negatively charged liposomes were employed. 1,6-Diphenyl-1,3,5-hexatriene depolarization measurements showed that the interaction of the peptide with both negatively charged and zwitterionic liposomes was accompanied by a substantial reduction of the transition amplitude without affecting the temperature of the gel-to-liquid crystalline phase transition. These data are indicative of the peptide insertion inside the bilayer of both types of liposomes affecting the acyl chain order, though only the interaction with acidic phospholipids leads to aggregation and fusion. This preferential destabilization of the peptide towards negatively charged phospholipids can be ascribed to the electrostatic interactions between the peptide and the polar head groups, as monitored by 1-(4-(trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene fluorescence depolarization analysis.

Keywords: Hepatitis B virus; Fusion peptide; Fluorescence depolarization; Electron microscopy

1. Introduction

The entry of enveloped viruses into their host cells requires the fusion between the viral and cellular plasma or endosomal membranes. In order to achieve the coalescence of the two bilayers, the majority of the best studied viruses possesses a clearly defined hydrophobic stretch of amino acids referred to as the fusion peptide [1,2]. In most cases, this fusion peptide is located at the N-terminus of one

Abbreviations: CD, circular dichroism; DMPC, dimyristoylphosphatidylcholine; DMPG, dimyristoylphosphatidylglycerol; DMPS, dimyristoylphosphatidylserine; DMSO, dimethyl sulfoxide; DPH, 1,6-diphenyl-1,3,5-hexatriene; HBV, hepatitis B virus; HIV, human immunodeficiency virus; PC, phosphatidylcholine; PS, phosphatidylserine; T_m, gel-to-liquid crystalline transition temperature; TMA-DPH, 1-(4-(trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene

* Corresponding author. Fax: +34 (91) 3944159;
E-mail: pacog@solea.quim.ucm.es

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of the envelope glycoproteins and results from the proteolytic processing of a polyprotein precursor. Sequence comparison among fusion peptides from different virus families reveals a few common features, such as the presence of the Phe-Leu-Gly tripeptide and a general high frequency of Gly residues, indicative of the conformational plasticity that they exhibit [1,2]. In the case of the well studied hemagglutinin protein (HA) from influenza virus, activation occurs by the development of the acidic pH of the endosome, triggering a massive conformational change that projects the fusion peptide into the membrane of the host cell. This fusion peptide is located at the N-terminal end of the HA2 subunit, and is responsible for overcoming the strong repulsive hydration, steric and electrostatic barriers that preclude the two membranes from coalescence and fusion. Interestingly, in the pre-activated, pre-fusogenic neutral pH state, the conformation of the fusion peptide consists of a series of sequential folds, similar to random coil, according to the high resolution crystallographic data available in the case of the HA glycoprotein [3,4]. However, after exposure of the hemagglutinin fusion peptide at acidic pH, its insertion inside the endosome membrane is accompanied by the adoption of an α-helical conformation that perturbs the bilayer integrity [5,6].

In the absence of an adequate in vitro system to analyze human hepadnavirus infection and perform site-directed mutagenesis studies, the initial infective steps of hepatitis B virus (HBV) remain elusive at present. Sequence comparison with amino-terminal fusion peptides from both the paramyxovirus and retrovirus families pointed out the possibility that the N-terminus of the S protein of HBV might be a fusion peptide [7]. A synthetic 16 amino acid peptide comprising this region was subsequently shown to interact with soybean asolectin phospholipids and to mediate liposome aggregation, lipid mixing and liposome leakage in a pH-dependent manner [8,9]. The close apposition of membranes and the destabilization of the bilayers needed to perform these effects take place only when negatively charged phospholipids are present [9]. Also, synthetic oligopeptides corresponding to the N-terminal region of the S protein of other representative hepadnaviruses possess these destabilizing properties [10]. The HBV putative fusion peptide adopts mainly a highly stable β-sheet structure when interacting with acidic and neutral phospholipid vesicles, as demonstrated by circular dichroism (CD) and Fourier transform infrared spectroscopy techniques [11]. The aim of this report is the characterization of the lipid moiety alteration upon addition of the fusion peptide by means of electron microscopy and fluorescence depolarization of two probes incorporated into the lipid bilayer. Our results suggest that the peptide is indeed fusogenic and although the peptide inserts in both negatively charged and zwitterionic phospholipid vesicles, electrostatic interactions between the fusion peptide and the polar head groups rather than hydrophobic forces might be responsible for the preferential aggregation and fusion observed towards acidic phospholipid vesicles.

2. Materials and methods

2.1. Chemicals

Egg phosphatidylcholine (PC), bovine brain phosphatidylserine (PS), dimyristoylphosphatidylcholine (DMPC), dimyristoylphosphatidylserine (DMPS) and dimyristoylphosphatidylglycerol (DMPG) were provided by Avanti Polar Lipids. 1,6-Diphenyl-1,3,5-hexatriene (DPH) and 1-(4-(trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH) were purchased from Molecular Probes. All other reagents were from Merck.

2.2. Peptide synthesis

A 16 amino acid peptide covering the amino-terminal region of the S protein of HBsAg (sequence: Met-Glu-Asn-Ile-Thr-Ser-Gly-Phe-Leu-Gly-Pro-Leu-Leu-Val-Leu-Gln) was synthesized as the carboxy-terminal amide on an automated multiple peptide synthesizer (AMS 422, Abimed) using a solid-phase procedure and standard Fmoc chemistry in a base of 25 μmol as previously described [9]. The peptide was purified by reverse-phase HPLC on a C-18 column. Purity and composition of the peptide were confirmed by reverse-phase HPLC and by amino acid analysis [9].

2.3. Electron microscopy

The peptide-vesicle complexes were obtained by incubating phospholipid vesicles (obtained by extrusion through a 0.1 mm pore diameter polycarbonate filter) [9] at 0.14 mM lipid concentration with peptide at a final concentration of 39 μM for 1 h at 37°C. Both the vesicles and the peptide-vesicle complexes were subjected to negative staining with phosphotungstic acid and observed in formvar-carbon grids. In general, the grid was subjected to a glow discharge in order to enhance its adherence properties and was immediately deposited over a drop of the sample for approx. 2 min. Afterwards, the grid was dried and stained with 1.7% (w/v) phosphotungstic acid, pH 7.2 for 1 min. Finally, the grid was dried and subjected to vacuum drying for 30 min. If the sample to be stained was at pH 5.0, an additional step of 1 min equilibration in medium buffer, pH 7.3, was included after incubation of the sample with the grid. Samples were examined using a Zeiss EM 902 (Jena, Germany) transmission electron microscope operating at 80 kV.

2.4. Fluorescence polarization

Two different probes were employed in the depolarization experiments: DPH and TMA-DPH. The former was dissolved in tetrahydrofuran and added to the lipid chloroform solution at a 1:500 ratio (w/w), and the latter was dissolved in methanol and added to the lipid chloroform solution at a 1:100 ratio (w/w). Lipid aliquots were dried under a nitrogen current for at least 30 min and were finally kept in vacuum overnight. Hydration was performed in 1.5 ml of medium buffer (100 mM NaCl, 5 mM Tris, 5 mM sodium citrate, 5 mM MES) adjusted to the appropriate pH value at 42°C for 1 h. The lipid vesicles were then sonicated for 30 min in a bath sonicator (Branson 1200) in order to obtain small unilamellar vesicles. The sample was divided into three 0.5 ml aliquots, each of them at 0.14 mM lipid. DMSO was added to one of these aliquots (control) and peptide from a concentrated DMSO stock solution was added to the other two (3.5–28 μM final concentration). The concentration of DMSO was kept below 1.5%. The three samples were measured at a time in 0.2×1 cm fluorescence cuvettes. A SLM Aminco 8000C spectrofluorimeter equipped with Glan Thompson polarizers was employed, setting the slits to 4 nm. The probes were excited at 365 nm and the fluorescence emission was measured at 425 nm. The temperature in the cuvette was maintained with a Polystat Huber circulating water bath.

3. Results

3.1. Electron microscopy

Previous data obtained with the HBV N-terminal peptide indicated that it was able to perform the essential steps required for fusion when acidic phospholipids were present in the assays but it was unable to induce these effects when added to neutral phospholipids even at elevated concentrations [8,9]. In order to inspect the changes induced in the liposome morphology upon addition of the N-terminal peptide, both natural phospholipid vesicles and vesicle-peptide complexes were observed by negative staining electron microscopy. Fig. 1 shows the control PS (A) and PC liposomes (C) negatively stained with phosphotungstic acid in the absence of added peptide. In both panels, a homogeneous population of vesicles, averaging 102 ± 20 nm (Fig. 1A) and 104 ± 20 nm (Fig. 1C), can be observed. Addition of the peptide to bovine brain PS liposomes led to an increase in vesicle size. Together with some original vesicles, large aggregates and fused structures of 240 ± 55 nm were observed (Fig. 1B). In the case of egg PC liposomes the vesicle size did not change significantly (100 ± 25 nm) upon addition of the peptide (Fig. 1D). Therefore, the N-terminal peptide of HBV induced the fusion of negatively charged phospholipid vesicles, but was unable to promote aggregation or fusion of neutral phospholipid liposomes.

3.2. Fluorescence depolarization measurements

Although not analogous to natural phospholipids, synthetic dimyristoyl phospholipids were used to assess the effect of the N-terminal fusion peptide on the lipid phase transition. Fluorescence depolarization of DPH and its polar derivative TMA-DPH incorporated into the bilayer was measured after interaction

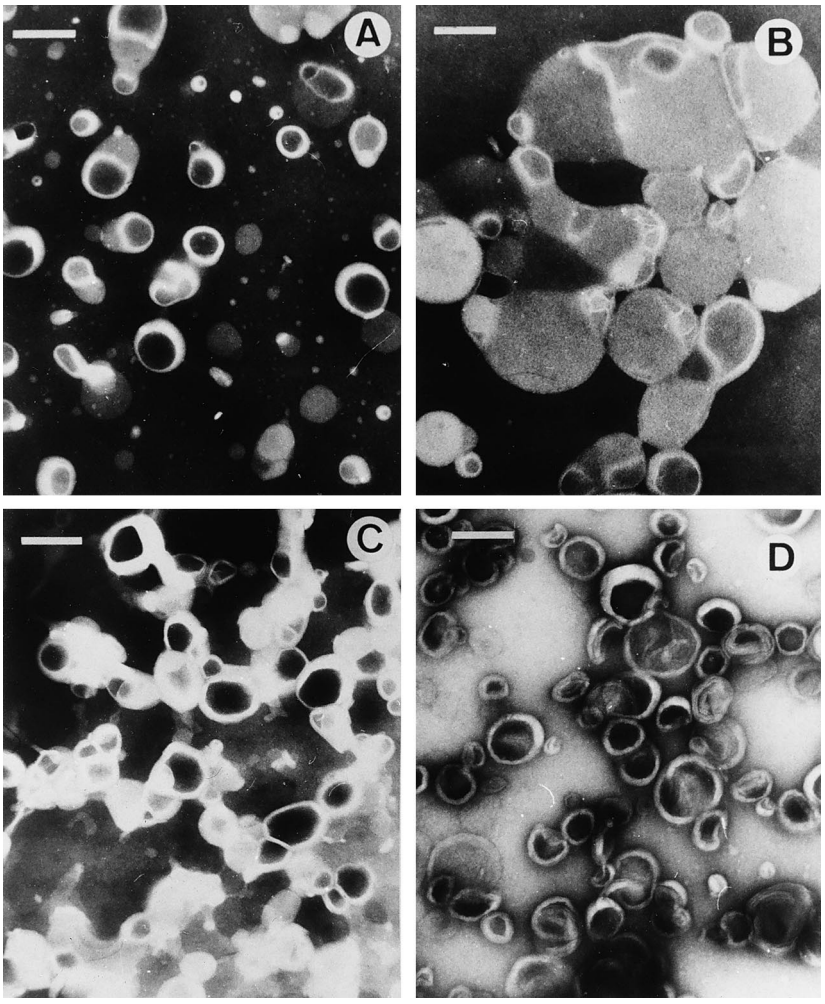

Fig. 1. Electron micrographs of liposomes and liposome-HBV peptide complexes. The complexes were obtained by incubating the vesicles (0.14 mM, final concentration) with the peptide (39 μM, final concentration) in medium buffer at pH 5.0 for 1 h at 37°C. Bovine brain PS vesicles in the absence (A) or presence (B) of the HBV peptide, and egg PC vesicles in the absence (C) or presence (D) of the HBV peptide were used to coat glow-discharged formvar-carbon grids as described in Section 2. The grids were subsequently stained with phosphotungstic acid. The bar represents 150 nm.

of the peptide with the vesicles for 1 h at 37°C. The two fluorescent probes were employed in the assays so that the fluidity of both the inner and the outer part of the bilayer could be monitored. DPH is assumed to be aligned with the phospholipid acyl chains giving information of the hydrophobic core of the bilayer, whereas TMA-DPH has a shallower location due to the anchoring of its non-fluorescent polar moiety to the lipid/water interface and interacts with both the phospholipid polar head groups and the fatty acyl chain region, probably as far down as C8-C10 [12–14]. Fig. 2 shows the fluorescence depolarization of DPH-labeled DMPS liposomes at both pH 7.3 and pH 5.0 upon interaction with the fusion peptide at two different concentrations. In the absence of added peptide, the DMPS vesicles displayed a cooperative transition, being the gel-to-liquid crystalline transition temperature (T_m) 35.5°C at pH 7.3 and 39°C at pH 5.0, in good agreement with published data [15,16]. This displacement in the DMPS phase transition towards higher temperatures together with the slight diminishment in the polarization amplitude, and hence in the transition enthalpy value, is due to the partial protonation of the serine carboxylate ($pK_{app} = 5.5$) [16]. Addition of the peptide modifies the phase transition curves of the

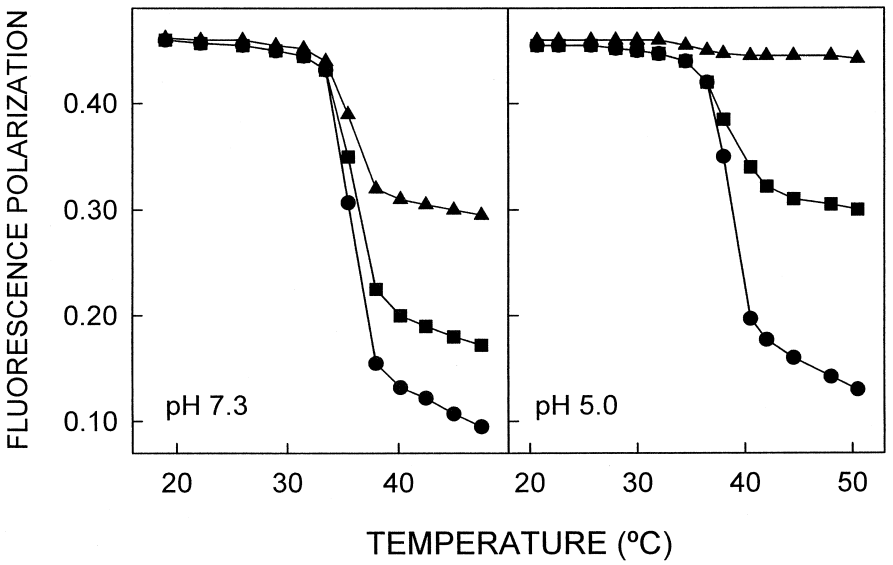

Fig. 2. Temperature dependence of the fluorescence polarization of DPH-labeled DMPS vesicles. Phosphatidylserine liposomes were prepared by hydration of a dry lipid film in medium buffer at pH 7.3 (left) or pH 5.0 (right) followed by bath sonication. The vesicles were incubated for 1 h at 37°C in the presence of 17.5 μM (■) or 28 μM (▲) HBV peptide dissolved in DMSO. In every case, a control experiment with DMSO alone (●) was also performed. After cooling the samples, fluorescence polarization was measured at the indicated temperature. The phospholipid concentration was 0.14 mM and the probe/phospholipid molar ratio was 1/500. The data are representative of at least three independent experiments.

vesicles both at pH 7.3 and pH 5.0. The amplitude of the phase transition decreased as a consequence of the increase in DPH fluorescence polarization of the liquid-crystalline state without significant changes in the transition temperature. The interaction of the peptide with the acyl chains scarcely affected the gel state, since the polarization values below the T_m remain unmodified. These effects on the thermotropic behavior of the phospholipid are those of an integral molecule which restricts the mobility of the acyl chains, suggesting the insertion of the peptide into the membrane. The modifications on the thermal transition induced by the peptide are significantly more profound at pH 5.0, with 28 μM peptide almost completely abolishing the gel-to-fluid phase transition (Fig. 2, right) while the same peptide concentration reduced the transition amplitude by 60% at pH 7.3 (Fig. 2, left). This increased interaction is likely due to the partial protonation of the side chain of the glutamic acid at position 2 of the peptide upon lowering the pH, allowing a deeper penetration within the bilayer.

Insertion of the peptide took place into acidic phospholipids with different polar head groups since a similar trend to that described for DMPS is observed when the peptide was added to DMPG vesicles, employing the probe DPH at both neutral and acidic pH (Fig. 3). Although no variation of T_m is observed, the amplitude of the gel-to-liquid crystalline transition decreased when the peptide was present. As described for the DMPS vesicles, the changes in the fluorescence polarization were observed only at temperatures above the T_m of the transition, indicative that the peptide preferentially alters the phospholipid acyl chain in its fluid state. Also, the effect observed at pH 5.0 was more profound than at pH 7.3. Moreover, at pH 5.0 the effect caused by the peptide is larger on the DMPG than on the DMPS vesicles since 17.5 μM peptide is enough to almost completely abolish the DMPG phase transition while that peptide concentration reduced the DMPS transition by 50%. This difference could be due to either a different partitioning of the peptide into these two phospholipids or to the reduced ability of phosphatidylglycerol, in comparison with phosphatidylserine, to form an extensive intermolecular hydrogen bond network that would prevent, to some extent, the peptide from disrupting the bilayer integrity [17].

As indicated by electron microscopy the fusion

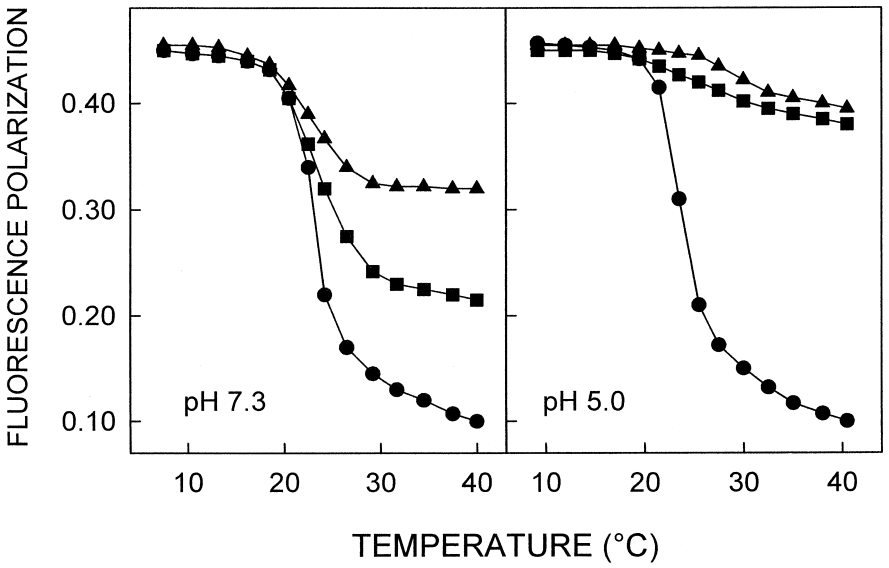

Fig. 3. Temperature dependence of the fluorescence polarization of DPH-labeled DMPG vesicles. The preparation of the vesicles and the rest of the conditions are as in Fig. 2. 17.5 μM (■) and 28 μM (▲) HBV peptide and control experiment with DMSO alone (●).

peptide was unable to induce neither aggregation nor fusion of egg PC liposomes. However, as depicted in Fig. 4, the phase transition of liposomes composed of DPH-labeled DMPC was also affected by the addition of the N-terminal peptide. Interaction occurred at the two pH values tested, with higher changes observed at pH 5.0. As observed for both DMPS and DMPG vesicles, the transition amplitude decreased while the T_m of the process remained constant, indicative of the insertion of the peptide into the core of the DMPC bilayer. A gradual decrease in the transition enthalpy was attained as the peptide concentration increased, although the complete cancellation of the phase transition was not observed in the concentration range tested. At 28 μM peptide concentration and pH 5.0 the transition amplitude

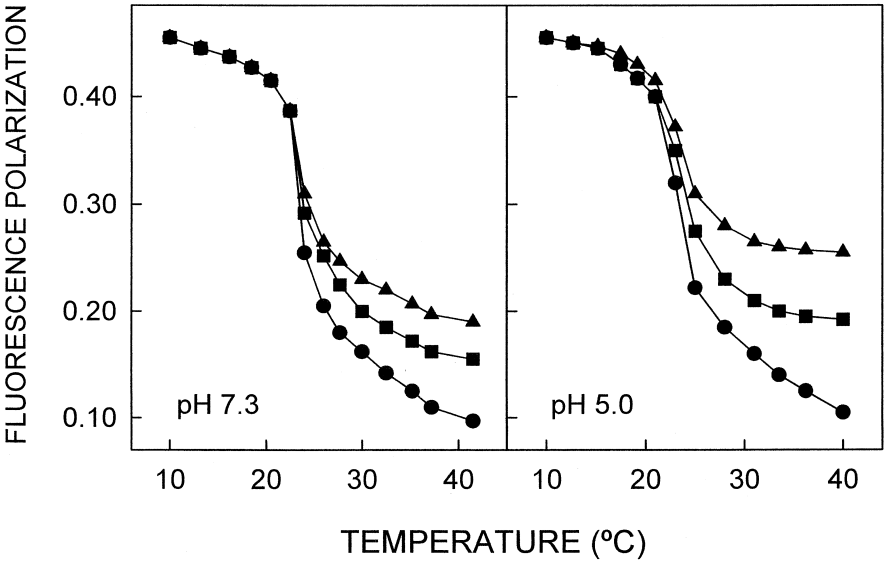

Fig. 4. Temperature dependence of the fluorescence polarization of DPH-labeled DMPC vesicles. The preparation of the vesicles and the rest of the conditions are as in Fig. 2. 17.5 μM (■) and 28 μM (▲) HBV peptide and control experiment with DMSO alone (●).

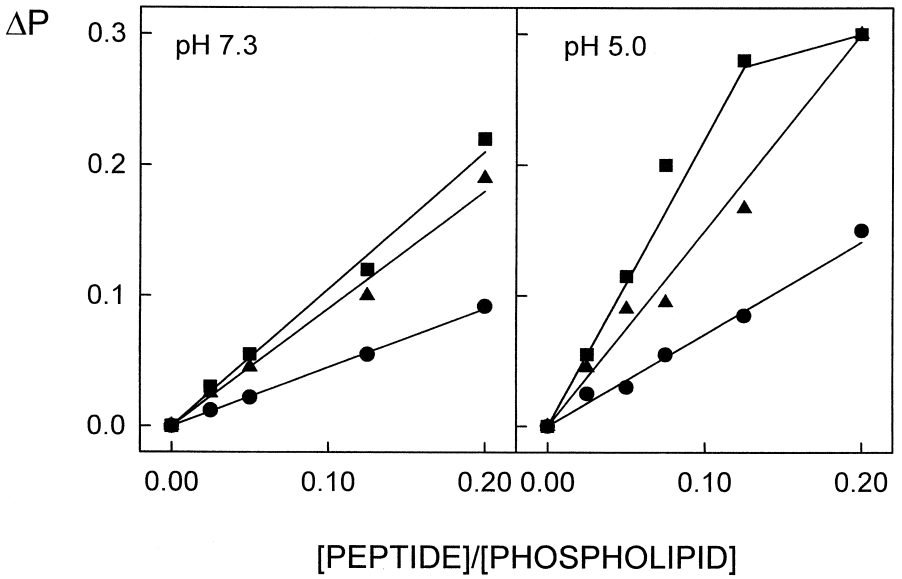

Fig. 5. Changes in the amplitude of the thermal transition of DPH-labeled vesicles with increasing peptide concentrations. At every peptide/phospholipid molar ratio, the ΔP value was obtained by subtracting from the fluorescence polarization value measured at maximal temperature the value of the control experiment performed in the presence of DMSO alone. Increasing amounts of the HBV fusion peptide dissolved in DMSO were added to DMPC (●), DMPS (▲) or DMPG (■) liposomes prepared at neutral (left) and acid (right) pH values. The mixtures were allowed to stand for 1 h at 37°C and the melting curve was recorded.

was reduced to 50%. Acidic pH values also augmented somehow the degree of interaction of the peptide with the liposomes, in accordance with the likely protonation of the glutamic acid at position 2 above mentioned. The insertion of the peptide was also assessed by differential scanning calorimetry (data not shown). The findings obtained are almost identical using either approach: the interaction with both acidic (DMPS) and zwitterionic (DMPC) vesicles is accompanied by the concomitant decrease in the enthalpy of the phase transition and with no noticeable changes in the T_m of the process.

In order to compare the effect of the peptide on the thermotropic behavior of the various types of phospholipids tested we have represented the changes in the amplitude of the thermal transition (ΔP) at increasing peptide/phospholipid molar ratios (Fig. 5). As defined, the bigger the effect on the phase transition the higher the ΔP value. Also a ΔP value close to 0.3 means an almost complete abolition of the gel-to-liquid crystal transition. As it can be observed in Fig. 5 at both pH values, there was a linear correlation between the changes induced in the transition amplitude and the increase in the amount of added peptide, with larger rates corresponding to pH 5.0 and with the relative order DMPG > DMPS > DMPC. In the case of DMPG at pH 5.0, abrogation of the phase transition was already achieved at a peptide/phospholipid ratio of about 0.125. The fact that larger ratios induced no additional changes in polarization would indicate that every peptide molecule prevents an average of 8–10 phospholipid molecules from undergoing the phase transition. Extrapolation of the linear increase to a ΔP value of 0.3 allowed the calculation of the number of phospholipid molecules affected by an individual peptide molecule in each case. Thus, depending on the pH value of the interaction, 2–3 DMPC molecules, 3–5 DMPS molecules or 4–10 DMPG molecules were perturbed by every peptide molecule.

The effect of the N-terminal peptide was also evaluated by fluorescence depolarization of TMA-DPH incorporated into DMPC and DMPS liposomes (Fig. 6). The addition of the peptide was reflected in changes in the thermotropic properties of surface regions of the bilayer of DMPS vesicles. When the fusion peptide was incubated with DMPC vesicles at concentrations as high as 28 μM, the alterations that were observed in the polarization of the TMA-

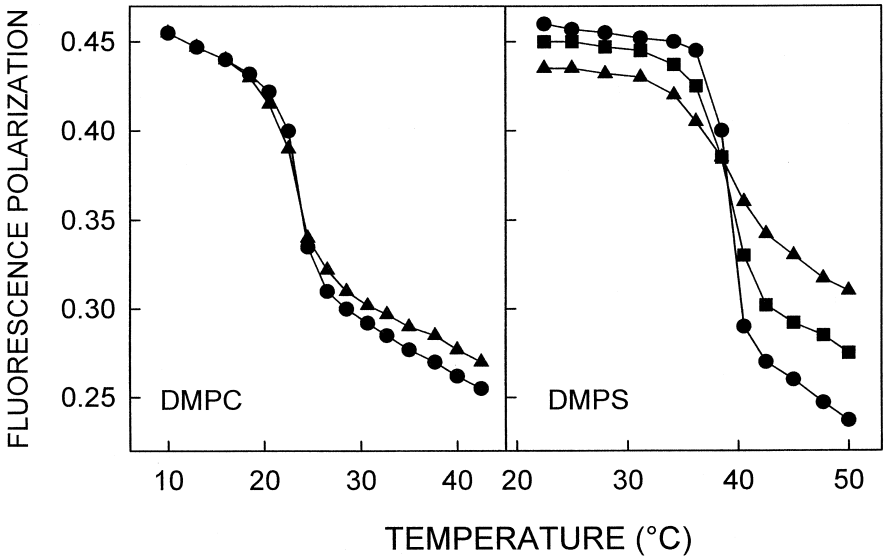

Fig. 6. Temperature dependence of the fluorescence polarization of TMA-DPH-labeled DMPC and DMPS vesicles. Liposomes were prepared in medium buffer at pH 5.0. The rest of the conditions are as in Fig. 2, except that the probe/phospholipid molar ratio was 1/100. 17.5 μM (■) and 28 μM (▲) HBV peptide and control experiment with DMSO alone (●).

DPH probe were almost negligible (Fig. 6, left). This is clearly not the case for the DMPS vesicles (Fig. 6, right) where the addition of the peptide clearly diminished the amplitude of the gel-to-fluid phase transition without promoting a modification in the T_m value. At 28 μM peptide the reduction of the transition amplitude was 35%, decreasing the order of the shallower region of the bilayer at temperatures below the T_m and increasing the order at temperatures above the T_m of the DMPS vesicles.

4. Discussion

Synthetic fusion peptides corresponding to the N-terminus of viral envelope glycoproteins have been widely used to determine the lipid destabilizing properties performed by these protein regions during the initial infective steps [1,2]. Detailed structural studies have been performed both in solution and in the presence of lipids. Accordingly, the different secondary structures adopted by the peptides as well as the different lipid compositions more suitable for aggregation, lipid mixing and fusion have been determined. Thus, both α-helix and β-sheet structures have been detected as the conformation of peptides in their fusion-active form, in numerous cases depending on the lipid composition and the lipid/peptide ratio ([1,2] and references therein). On the other hand, the conformational plasticity of the fusion peptide together with its membrane-destabilizing properties, rather than a certain structural conformation, appear to be the common determinants of these N-terminal extensions [1,18].

Previous experiments performed in our laboratory have shown that peptides derived from the N-terminal region of three different members of the hepadnavirus family destabilize model membranes efficiently. They are flexible enough to adopt different conformations when challenged in different environments and both extended and helical conformations seem to be responsible for the destabilizing properties [9–11]. In the case of the HBV N-terminal peptide stepwise sucrose gradient centrifugation allowed to measure binding to soybean asolectin phospholipid vesicles. After incubation at pH 5.0 for 2 h at 37°C and a peptide/phospholipid molar ratio of 0.111 approx. 40% of the peptide was bound to the vesicles [8]. Moreover, the peptide was able to induce aggregation, lipid mixing as monitored by a fluorescent probe dilution assay and release of aqueous contents of bovine brain PS or soybean asolectin phospholipid vesicles. However, these effects could not be observed when egg PC liposomes were used [9]. Although these destabilizing properties are usually considered the essential steps required for fusion [19], no direct

evidence for it had been reported. Electron microscopy studies performed under conditions in which part of the peptide is bound to the vesicles show that the N-terminal peptide is indeed fusogenic but only towards negatively charged phospholipids. This specificity points to the electrostatic interactions as a key factor in eliciting the destabilization. Concerning the preferences of fusion peptides towards the lipid composition of the target membrane a great diversity has been reported. Thus, an N-terminal fusion peptide of influenza virus hemagglutinin was found to preferentially interact with PC rather than PS vesicles whereas the fusion peptide of vesicular stomatitis virus destabilized PS rather than PC liposomes [20]. More striking appeared to be the lipidic requirements of the Ebola virus fusion peptide, where phosphatidylinositol rather than any other acidic phospholipid was required in order to induce membrane fusion [21]. A simple explanation to this behavior might be found in the underlying tendency of the peptide sequences to adopt a certain conformation in the fusogenic state. Nevertheless, according to the CD and Fourier transform infrared spectroscopy data, the HBV fusion peptide adopted a β-sheet conformation in the presence of both neutral and acidic phospholipids [11]. Hence, some other factor must account for the specificity towards negatively charged vesicles.

The modification of the lipid-lipid interaction induced by the HBV peptide under conditions that induce aggregation and fusion was measured by means of fluorescence depolarization. The data obtained with the probe DPH showed that the HBV fusion peptide inserted deeply within the DMPS and DMPG bilayers, affecting the gel-to-liquid crystal transition by diminishing the amplitude value without changing the T_m. These results point out the relevance of the hydrophobic interaction in the association of the peptide with the vesicles. On the other hand, lowering the pH of the buffer resulted in an increased interaction with liposomes, very likely as a consequence of the partial protonation of glutamic acid at position 2 in the sequence of the peptide. Although the partial protonation of the carboxylate of DMPS might be envisioned as the reason for this improved interaction at acidic pH, the fact that at pH 5.0 there is a very significant augmentation in the interaction with DMPG vesicles suggests that a change in the protonation state of the peptide, rather than the lipid itself might be responsible for this trend.

According to the DPH polarization experiments, the fusion peptide was also able to insert within the membrane interior of DMPC vesicles, decreasing the enthalpy of the transition without inducing changes in the value of the T_m. It must be remarked that under the maximal concentration of peptide used in the polarization studies (28 μM) neither vesicle aggregation, lipid mixing nor leakage of egg PC liposomes was observed, regardless of the pH of the assay [9]. Moreover under the light of the electron microscopy results, this insertion of the peptide within the acyl moiety of the PC bilayer does not result in vesicle fusion or aggregation, not even at concentrations as high as 39 μM. On the other hand, when DMPS vesicles were labeled with TMA-DPH, changes in the fluidity of the membrane both below and above the T_m of the transition were observed, indicative that the peptide is disrupting a more superficial part of the bilayer. However, the peptide caused an almost negligible disruption of that region of the DMPC bilayers. Thus, fusion is only observed when the peptide is able to interact with both the polar head groups and the acyl chains, while alterations of only the acyl chains do not result in membrane fusion. This correlation between the fusion peptide membrane penetration and its ability to promote aggregation and fusion has also been reported for human immunodeficiency virus (HIV) and Sendai virus fusion peptides [22,23]. In these two cases, both wild-type and mutant fusion peptides were labeled with the fluorescent reagent NBD and their penetration within the bilayer was monitored. Whereas the fusion-defective V2E mutant of HIV was inserted deeper than the wild-type peptide inside the bilayer, the more fusogenic G12A fusion peptide mutant of Sendai virus was found to arrange closer to the surface of the membrane than the wild-type [20,24]. Therefore, there seems to be a general correlation and in fact, it has been stated that a shallow penetration of a fusion peptide is likely responsible for the destabilization of the lipids required for coalescence of the membranes and fusion [1].

The use of attenuated total reflection Fourier-transform infrared spectroscopy has allowed to determine the orientation with respect to the bilayer

plane of fusion peptides that insert in the bilayer in an α-helical conformation. An oblique orientation has been found to be a prerequisite for fusion of viral peptides from simian immunodeficiency virus [24,25], HIV [26] and influenza virus [27,28] as well as a peptide derived from fertilin, a protein active in sperm-egg fusion [29]. However, when the peptide display an extended β-structure, no orientation can be experimentally determined [2]. Only indirect evidence for an oblique orientation of HIV gp41-derived peptides has been reported [23]. The fact that HBV fusion peptide is able to disrupt both the polar head group and the acyl chains of acidic phospholipids would point to an oblique mode of insertion while a perpendicular insertion in neutral phospholipids would only affect the hydrophobic core of the bilayer. Also, the oblique insertion of HBV fusion peptide in negatively charged phospholipids would account for the higher number of phospholipid molecules that the peptide prevents from undergoing the phase transition as compared with neutral phospholipids. Accordingly, HBV and other β-sheet fusion peptides may also induce the negative curvature that favors the formation of inverted phases which seem to be involved in fusion [25,29].

Acknowledgements

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