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Effect of magainin, class L, and class A amphipathic peptides on fatty acid spin labels in lipid bilayers

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Abstract

Magainins and other antimicrobial peptides increase ion flux across the membrane. They may do this by forming some type of pore or by perturbing lipid organization due to peptide lying on the bilayer surface. In order to determine if magainins perturb the lipid sufficiently to permeabilize the bilayer, their effect on the motion of fatty acid and lipid spin labels in phosphatidylcholine/phosphatidylglycerol (PC/PG) lipid vesicles was determined. Their effect was compared to two synthetic peptides, 18L and Ac-18A-NH_2, designed to mimic the naturally occurring classes of lytic (class L) and apolipoprotein (class A) amphipathic helices, respectively. We show that although magainins and 18L both had significant effects on lipid chain order, much greater than Ac-18A-NH_2, there was no correlation between these effects and the relative ability of these three peptide classes to permeabilize PC/PG vesicles in the order magainins = Ac-18A-$NH_2 \gg$ 18L. This suggests that the perturbing effects of magainins on lipid chain order at permeabilizing concentrations are not directly responsible for the increased leakage of vesicle contents. The greater ability of the magainins to permeabilize PC/PG vesicles relative to 18L is thus more likely due to formation of some type of pore by magainins. The greater ability of Ac-18A-NH_2 relative to 18L to permeabilize PC/PG vesicles despite its lack of disordering effect must be due to its ability to cause membrane fragmentation. Effects of these peptides on other lipids indicated that the mechanism by which they permeabilize lipid bilayers depends both on the peptide and on the lipid composition of the vesicles.

Abbreviations: PE, phosphatidylethanolamine; POPG, 1-palmitoyl-2-oleoylphosphatidylglycerol; DPPG, dipalmitoylphosphatidylglycerol; POPE, 1-palmitoyl-2-oleoylphosphatidylethanolamine; 5-S-SL, 5-doxyl-stearic acid; 12-S-SL, 12-doxyl-stearic acid; 16-S-SL, 16-doxyl-stearic acid; PG-14-S-SL, 1-palmitoyl-2-(14-doxylstearoyl)-*sn*-glycero-3-phosphoglycerol; Mag, Magainin 2 amide (GIGKFLHSAKKFGKAFVGEIMNS-amide); Ala_{19}-Mag, Ala_{19}-magainin 2 amide (Mag with Ala substituted for Glu_{19}); 18L, GIKKFLGSIWKFIKAFVG; Ac-18A-NH_2, N-acetyl-DWLKAFYDKVAEKLKEAF-amide; T_H, bilayer to hexagonal phase transition temperature; ANTS, aminonaphthalene-3,6,8-trisulfonic acid; DPX, *p*-xylenebis(pyridinium bromide); MLVs, multilamellar vesicles; LUVs, large unilamellar vesicles

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1. Introduction

Magainins are antimicrobial, non-hemolytic peptides isolated from the skin of the African clawed frog, *Xenopus laevis* [1–3]. Their antimicrobial activity may be due to their ability to permeabilize negatively charged membranes and lipid vesicles to ions [2,4–6]. The mechanism by which they permeabilize lipid bilayers is not clear, however. Electrophysiological studies indicated that magainin increases ion flux across the membrane [7–10]. It may be able to do this by forming a pore which would require location of a portion of the lipid-associated peptide within the bilayer.

Although many studies of the orientation and topography of magainin in lipid bilayers have been carried out, these have yielded conflicting results [11–17]. The kinetics of permeabilization of lipid vesicles containing phosphatidylglycerol (PG) induced by magainin suggests that if pores are formed, they are transient and are followed by translocation of the peptide to the inner leaflet of the bilayer [12,18]. The transient nature of the pore and its low concentration may account for the fact that spectroscopic techniques have not been able to detect the existence of magainin pores [15–17]. However, magainin-induced ^2H_2O-containing pores were detected by neutron scattering [14]. A toroidal model in which the peptides lining the pore are bound to the head groups of lipid molecules oriented parallel to the bilayer was suggested to account for the fact that translocation of magainin was accompanied by lipid flip-flop across the bilayer [19] and for the large size of the water pore detected by neutron scattering [14]. Pore formation by at least a fraction of the peptide bound to the lipid bilayer was supported by our lipid photolabel study which showed that magainin could be labeled in PG-containing lipid vesicles by a photolabel group located at the end of a lipid acyl chain, and that labeling increased with peptide to lipid (P/L) ratio, indicating a cooperative effect [20]. The latter is consistent with a sigmoidal peptide concentration dependence of permeabilization of PG-containing lipid vesicles [8,11]. Pore formation has also been detected by high sensitivity titration calorimetry [21]. Taken together, previous studies indicate that most of the peptide lies on the bilayer surface but permeabilization of PG-containing vesicles is due to pore formation by a small percentage of the peptide.

However, an alternative mechanism for magainin-induced permeabilization of lipid vesicles is by perturbation of lipid packing by peptide lying on the surface of the bilayer with the hydrophobic side chains of the helix dipping into the bilayer, e.g. as in the in-plane diffusion model described by Bechinger [22]. Molecular dynamics calculations indicate that a lytic peptide such as melittin lying on the surface of the bilayer can perturb the lipid in the vicinity of the peptide such that penetration of water into the bilayer is favored [23]. In fact, the mechanism of permeabilization by magainin may be dependent on lipid composition. Although permeabilization of lipid vesicles containing PG was dependent on the P/L ratio in a sigmoidal fashion, indicating a cooperative effect which supported pore formation, permeabilization of lipid vesicles containing phosphatidylserine (PS), phosphatidic acid (PA), and cardiolipin (CL) increased with P/L ratio in a non-sigmoidal fashion and required 10–20 times higher magainin to lipid ratio for permeabilization than vesicles containing PG [24]. This was attributed to the ability of the former lipids to go into the hexagonal II phase or experience negative curvature strain when their negatively charged head groups are neutralized by electrostatic binding of divalent cations. Possibly positively charged peptides such as magainins have a similar effect. Induction of negative curvature strain by the peptide would destabilize a toroidal type of pore in which high positive curvature of the lipid occurs in a dimension perpendicular to the bilayer plane [24]. The higher concentration of magainin required for permeabilization of vesicles containing PS, PA, and CL and the lack of peptide cooperativity suggests that these lipid vesicles may be permeabilized by surface-bound peptide rather than pores. At high concentrations, magainin also permeabilizes phosphatidylcholine (PC) vesicles in a

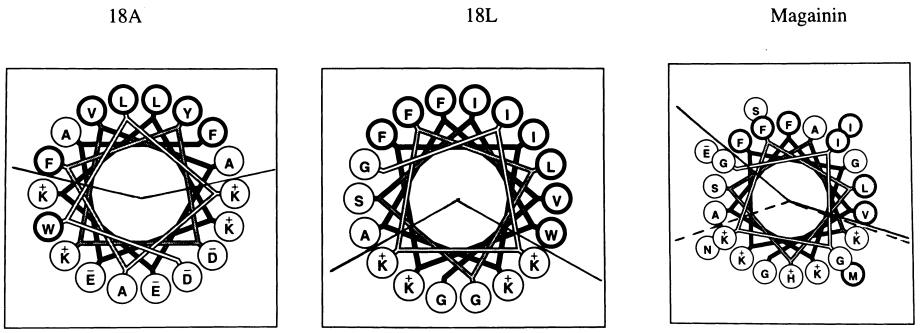

Fig. 1. Helical wheel diagrams of (A) 18A, (B) 18L, and (C) Mag. Ala_{19}-Mag has Ala substituted for Glu at position 19. Hydrophobic amino acids are outlined in bold. The angle subtended by the apolar face is indicated. In C the angle for Mag is indicated by a continuous line and that for Ala_{19}-Mag is indicated by a dashed line.

non-sigmoidal fashion, possibly by a similar mechanism [25].

Even if the peptide lying on the bilayer surface results in only transient formation of defects in the bilayer, the occurrence of such defects should be proportional to the ability of the peptide to alter lipid chain packing. This can be measured by spectroscopic studies of lipid chain order or by measuring the effect of the peptide on lipid properties such as its gel to liquid crystalline phase transition. However, little is known of the effect of magainins on lipid chain order and packing. In the present study we have examined the effect of magainin 2 amide (Mag) and Ala_{19}-magainin 2 amide (Ala_{19}-Mag) on the motion of fatty acid spin labels in lipid vesicles. Ala_{19}-Mag is a more antimicrobial analogue of Mag in which Ala replaces Glu at position 19 [17,26]. We compare the effects of the magainins on lipid chain order and on vesicle permeability with the action of two synthetic peptides, 18L and Ac-18A-NH_2, designed to mimic the naturally occurring classes of lytic (class L) and apolipoprotein (class A) amphipathic helices, respectively [27]. The class A amphipathic helix has a wide polar face containing clusters of positively charged residues at the polar/apolar interface and a cluster of negatively charged residues at the center of the polar face (Fig. 1). The class L amphipathic helix has a narrower polar face containing predominantly positively charged residues and its apolar face has a number of bulky hydrophobic amino acids such as Ile and Phe [28]. The magainins have been assigned to the class L type on the basis of the size and charge distribution of their hydrophilic domains. However, unlike the magainins, 18L does not appear to form pores [29]. Furthermore, 18L destabilizes the lipid bilayer relative to the hexagonal H_{II} phase while magainins stabilize it. The stabilizing effect of magainin on the lamellar phase is similar to the effect of Ac-18A-NH_2, as indicated by their effects on the temperature of the phase transition from bilayer to hexagonal phase (T_H) [24,27]. These differences in behavior may be partly due to a dependence of peptide effect on the lipid composition [24,29] and partly to the fact that 18L has a wider hydrophobic face (larger angle subtended by its hydrophobic face) and a greater number of bulky hydrophobic amino acids than magainin [27,30].

We show that there was no correlation between the relative effects of these three peptide classes on lipid chain order and their abilities to permeabilize PG-containing lipid vesicles. The magainins and 18L had a significant perturbing effect on lipid chain order but much higher P/L ratios for 18L were required to permeabilize lipid vesicles than for the magainins. Ac-18A-NH_2 permeabilized PG-containing lipid vesicles almost as effectively as the magainins but had little effect on lipid chain order. This suggests that the perturbing effect of magainins on lipid chain order which is observed at permeabilizing P/L ratios is not responsible for the increase in permeability. Thus the ability of the magainins to permeabilize PG-containing vesicles is more likely due to formation of some type of pore. The greater ability of Ac-18A-NH_2 relative to 18L to permeabilize PG-containing vesicles is probably due to its ability to fragment lipid vesicles into discs [31]. However, at higher P/L ratios, where 18L also permeabilizes PG-containing vesicles, and the magainins permeabilize vesicles

containing PS, PA, or CL, the permeabilization may be due to the perturbation of lipid chain order by these peptides lying on the surface of the bilayer. The greater ability of Ac-18A-NH_2 to permeabilize PG-containing vesicles compared to 18L was contrary to expectation, based on the reported effects of these peptides on vesicles containing phosphatidylethanolamine (PE) [27,29]. The latter has been correlated with the ability of these peptides to affect intrinsic lipid monolayer curvature and destabilize the bilayer. Thus the mechanism by which peptides permeabilize lipid bilayers depends both on the peptide and on the lipid composition of the vesicles.

2. Materials and methods

2.1. Lipids and peptides

Egg L-α-phosphatidylcholine (PC) was purchased from Sigma (St. Louis, MO). L-α-Phosphatidylglycerol (PG), prepared enzymatically from egg PC, dipalmitoyl-L-α-phosphatidylglycerol (DPPG), palmitoyloleoyl-L-α-phosphatidylglycerol (POPG), and palmitoyloleoyl-L-α-phosphatidylethanolamine (POPE) were obtained from Avanti Polar Lipids (Alabaster, AL). 5-Doxyl-stearic acid (5-S-SL), 12-doxyl-stearic acid (12-S-SL), and 16-doxyl-stearic acid (16-S-SL) were purchased from Aldrich. 1-Palmitoyl-2-(14-doxyl-stearoyl)-*sn*-glycero-3-phosphoglycerol (PG-14-S-SL) was a kind gift from Dr. A. Watts (University of Oxford). Mag (GIGKFLHSAKKFGKAFVGEIM-NS-amide) and Ala_{19}-Mag in which Ala is substituted for Glu at position 19 were synthesized using FMOC chemistry and purified by reverse-phase HPLC [17]. The amphipathic peptides Ac-18A-NH_2 (*N*-acetyl-DWLKAFYDKVAEKLKEAF-amide) and 18L (GIKKFLGSIWKFIKAFVG) [32] were synthesized using an ABI433A peptide synthesizer and FMOC chemistry. The peptides were cleaved from the resin using TFA in the presence of anisole and 1,2-ethanedithiol as scavengers. The peptides were purified by reverse phase HPLC [33]. Aminonaphthalene-3,6,8-trisulfonic acid (ANTS) and *p*-xylenebis(pyridinium bromide) (DPX) were purchased from Molecular Probes (Junction City, OR). Lubrol was purchased from Calbiochem (San Diego, CA).

2.2. Preparation of multilamellar vesicles for EPR spectroscopy

Aliquots of chloroform/methanol solutions of PC, PG, and spin label were combined to give the PC/PG/spin label mole ratio of 4:1:0.05. POPE or DPPG solutions were combined with spin label solutions in chloroform/methanol to give the lipid/spin label mole ratio of 1:0.01. The organic solvent was evaporated under a stream of nitrogen. In order to achieve homogeneous mixing of the lipids, the dry lipid film was redissolved in 1–2 ml of benzene, frozen, and lyophilized overnight. Multilamellar vesicles (MLVs) were prepared by hydrating the dry lipid (0.5–1 mg) in 0.2 ml of 10 mM HEPES buffer, pH 7.4, containing 150 mM NaCl and 0.5 mM EDTA. The required volume of peptide solution (0.5–1.0 mg/ml) prepared in the same buffer was added and additional buffer was added to make the final volume 0.5 ml. The lipid was dispersed by vortexing the suspension for 15 min at 45°C for DPPG and at room temperature for PC/PG and POPE. The samples were centrifuged in an Eppendorf bench centrifuge for 5 min. All but about 50 μl of supernatant was removed and the suspension was loaded into a 50 μl capillary tube. Since Ac-18A-NH_2 clarified the DPPG sample, it was concentrated for EPR measurement by centrifugation in a Microcon 3 centrifugal concentrator (Millipore, Milford, MA) at $13\,800 \times g$ for 90 min at 4°C. The remaining suspension from two Microcons was combined (40 μl) and loaded into a 50 μl capillary tube. Negative staining electron microscopy showed that it contained fragmented membranes of varying sizes (not shown). The capillary tubes were sealed at one end with a torch and the tube was centrifuged at 2000 rpm for 10 min.

2.3. Measurement of EPR spectra

EPR spectra were measured on a Varian E-104B EPR spectrometer equipped with a Varian temperature controller and a DEC LSI-11 based microcomputer system. DPPG samples were measured at various temperatures from 7 to 52°C on heating by raising the temperature and on cooling by lowering the temperature. PC/PG samples were measured at 21–37°C and POPE at 29–44°C. The order parameter *S* was determined from the anisotropic hyperfine

splittings, T_{perp} and $T_{//}$, and the motional parameter τ_o was measured as described earlier [34]. For some samples containing peptide, a small fraction of the spin label was present in the aqueous phase giving an isotropic spectrum which overlapped with some of the peaks from the membrane-bound spectrum. In those cases, T_{perp} was measured, since the isotropic spectrum did not interfere with those peaks. T_{perp} decreases with increase in the order parameter. In some cases, the motion was not sufficiently isotropic to measure τ_o since the high field peak was broad and asymmetric. In those cases, the ratio of intensities of the center to low field lines (h_0/h_{+1}) was measured. This ratio increases with decrease in motion as the spectrum becomes more asymmetric. It was also measured for samples where little change in τ_o occurred, since it can be measured more accurately. ΔS, ΔT_{perp}, $\Delta\tau_o$, and $\Delta h_0/h_{+1}$ were calculated by subtracting the value for the lipid alone from that of the lipid-peptide sample. Positive values indicate a peptide-induced increase in order or decrease in motion except for ΔT_{perp}. Therefore, $-\Delta T_{perp}$ was plotted in the figures. One half of the maximum hyperfine splitting, T_{max}, measured as shown earlier [35] was used as a measure of the degree of motional restriction of the probe in the lipid gel phase. The effects of Ala_{19}-Mag, 18L, and Ac-18A-NH_2 on the gel to liquid crystalline phase transition temperature of DPPG were monitored from the change in height of the center line with temperature of the EPR spectrum of 16-S-SL, since it increases with increased mobility of the spin label.

2.4. Preparation of large unilamellar vesicles and permeability measurements

The effect of the peptides on vesicle permeability was measured by the ANTS/DPX assay [36] from the dequenching of ANTS released into the medium as described earlier [29]. Large unilamellar vesicles (LUVs) of PC/POPG 4:1 were prepared as above except that the dry lipid film was hydrated with HEPES/MES/citrate buffer at pH 7.4, containing 12.5 mM ANTS and 45 mM DPX adjusted to 310 mOsm. LUVs were prepared by repeated extrusion of MLVs through two stacked 100 nm pore polycarbonate filters (Nucleopore, Pleasanton, CA). Vesicles with encapsulated contents were separated from the media on a Sephadex G-75 column equilibrated with HEPES/MES/citrate buffer at pH 7.4, osmolarity adjusted to 310 mOsm to balance the osmotic strength. Leakage was monitored from the increase in fluorescence intensity at 530 nm using 350 nm excitation and bandwidths of 4 and 8 nm for excitation and emission, respectively. A 490 nm cutoff filter was used between the monochromator and the cuvette holder to diminish scattered light. Leakage was initiated by injection of a vesicle suspension into a cuvette containing 2 ml of the peptide solution. Fluorescence was scanned for 600 s at 37°C on an SLM AB-2 fluorescence spectrophotometer. The lipid and peptide concentrations were 50 μM and 2.5 μM, respectively. Similar results were obtained at 20 μM lipid and 1 μM peptide concentrations. The 100% leakage level was determined by addition of 25 μl of 20% Lubrol. All runs were done in duplicate at 37°C with continuous magnetic stirring.

3. Results

3.1. Effect of peptides on permeability of PC/POPG vesicles

At a P/L mole ratio of 0.05, Ala_{19}-Mag, Mag, and Ac-18A-NH_2 caused rapid leakage of ANTS from PC/POPG 4:1 (mole ratio, m/m) vesicles. The rate of leakage decreased in the order Ala_{19}-Mag > Mag > Ac-18A-NH_2 (Fig. 2). However, 18L caused only a small amount of leakage at this P/L ratio. The extent of leakage after 500 s was comparable for Ala_{19}-Mag and Mag, about 80%; it was 60% for Ac-18A-NH_2 and 12% for 18L. P/L ratios of 0.1 or greater were necessary in order for 18L to cause over 50% leakage of PG-containing vesicles (not shown). Mag caused some leakage at a P/L ratio as low as 0.0095 (not shown).

3.2. Effect of peptides on fatty acid spin labels in PC/PG vesicles

In PC/PG 4:1 vesicles, Ala_{19}-Mag, Mag, and 18L all increased the order or decreased the amplitude of motion of fatty acid or lipid spin labels with the nitroxide at carbons 5, 12, 14, and 16 of an 18 carbon chain, used to monitor different positions

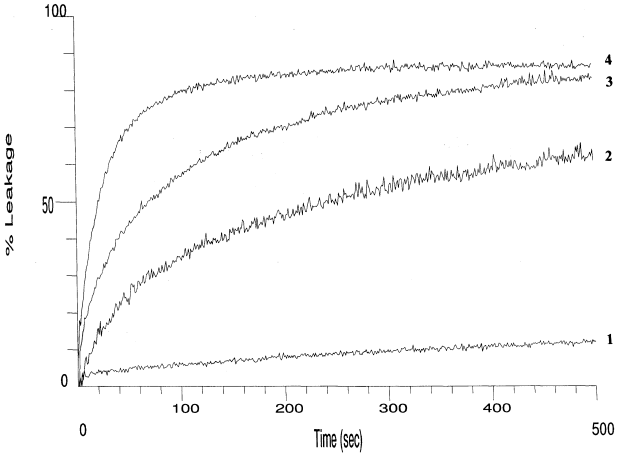

Fig. 2. Typical time dependence of peptide-induced leakage of aqueous contents of PC/POPG 4:1 LUVs using the ANTS/DPX leakage assay. Lipid and peptide concentrations were 50 μM and 2.5 μM, respectively. P/L mole ratio is 0.05 (m/m). 1, 18L; 2, Ac-18A-NH_2; 3, Mag; 4, Ala_{19}-Mag.

throughout the bilayer (Figs. 3 and 4). These spin labels were 5-S-SL, 12-S-SL, PG-14-S-SL, and 16-S-SL. The effect increased with increase in P/L ratio and P/L ratios of 0.04 or more were required to cause large effects. The increased order parameter of 5-S-SL and decrease in T_{perp} (increase in $-T_{perp}$) of 12-S-SL and PG-SL indicate increased order or reduced amplitude of motion of these probes while the small increase in τ_o of 16-S-SL indicates decreased motional frequency of this probe. Thus these peptides affected the lipid acyl chains at all depths in the bilayer with the effect significantly decreasing toward the center of the bilayer. 18L had the largest effect followed by Ala_{19}-Mag and then Mag down to carbon 16. However, Ac-18A-NH_2 had much less effect on the motion of the spin labels in PC/PG vesicles (Fig. 4). The effects of the magainins and 18L are attributed to the peptide amphipathic α-helix lying on the surface of the bilayer with its hydrophobic surface and bulky amino acid side chains dipping partway into the bilayer. The low effect of Ac-18A-NH_2 on 5-S-SL is especially notable since this position in the bilayer is most sensitive to the effects of surface-bound peptides.

The binding constants of these peptides to dioleoylphosphatidylglycerol at 12.5 μM peptide concentration have been reported. They are similar for Ac-18A-NH_2 and Mag (3.5×10^6 M^{-1} [37] and 3×10^6 M^{-1} [38], respectively) while that of 18L is 10 times higher (4.5×10^7 M^{-1} [37]). Thus differences in binding are not likely to account for differences in effect of these peptides on the permeability and chain order of PC/PG 4:1 vesicles.

It should also be noted that no spectral component characteristic of significantly restricted motion, typical of boundary lipid around transmembrane pro-

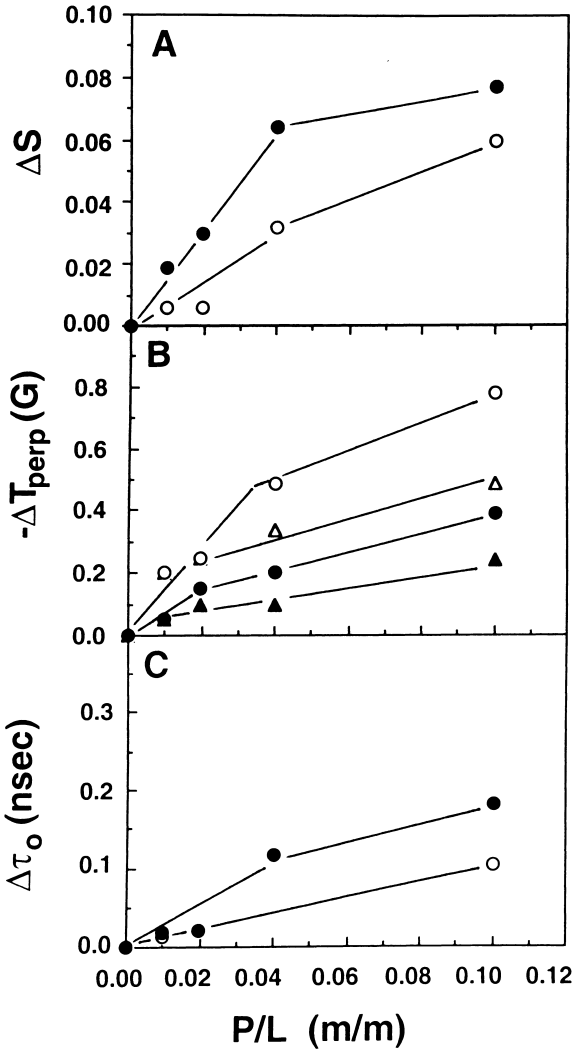

Fig. 3. Effect of magainin peptides on EPR parameters of spin labels in PC/PG 4:1 vesicles at 21°C. Change in parameters with increase in P/L ratio for (A) ΔS of 5-S-SL for Mag (○) and Ala_{19}-Mag (●); (B) $-\Delta T_{perp}$ of 12-S-SL for Mag (△) and Ala_{19}-Mag (○), and PG-14-S-SL for Mag (▲) and Ala_{19}-Mag (●); (C) $\Delta\tau_o$ of 16-S-SL for Mag (○) and Ala_{19}-Mag (●). Note that in B and C some data points for the two peptides at low P/L ratios coincide. The accuracy of measurement of the changes is ±0.01 for ΔS, ±0.1 G for ΔT_{perp}, and ±0.025 ns for $\Delta\tau_o$.

teins [39], was observed for 12-S-SL, 16-S-SL, or PG-SL even at a P/L ratio of 0.1 for any of the peptides. This indicates that if the peptides formed pores by transmembrane orientation, they either did not restrict the motion of lipid acyl chains or the transmembrane pore-forming fraction was present in too low a concentration to affect a detectable percentage of the probe.

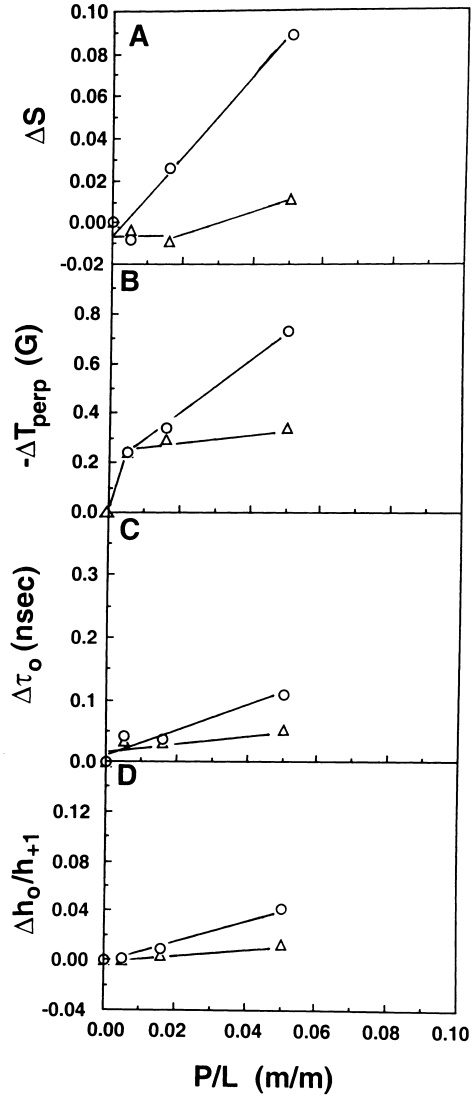

Fig. 4. Effect of Ac-18A-NH_2 (△) and 18L (○) on EPR parameters of spin labels in PC/PG 4:1 vesicles at 37°C. Change in parameters with increase in P/L ratio for (A) ΔS of 5-S-SL; (B) $-\Delta T_{perp}$ of 12-S-SL; (C) $\Delta\tau_o$ of 16-S-SL; and (D) $\Delta h_0/h_{+1}$ of 16-S-SL. Note that some data points for the two peptides at high P/L ratios coincide. The accuracy of measurement of the changes is ±0.01 for ΔS, ±0.1 G for ΔT_{perp}, ±0.025 ns for $\Delta\tau_o$, and ±0.01 for $\Delta h_0/h_{+1}$.

3.3. *Effect of Ac-18A-NH_2 and 18L on fatty acid spin labels in POPE vesicles*

18L and Ac-18A-NH_2 also bind to PE in contrast to magainins, which bind much less to zwitterionic lipids than to acidic lipids. In contrast to the greater effect of Ac-18A-NH_2 compared to 18L on the permeability of vesicles containing PG, these two peptides have opposite effects on the permeability of vesicles containing PE and on the T_H of PE [27,29]. 18L increases the permeability of PC/PE vesicles and decreases T_H, while Ac-18A-NH_2 decreases the 18L-induced permeabilization of PC/PE vesicles and increases T_H. This suggests that modulation of negative intrinsic monolayer curvature and destabilization of the bilayer are involved in the 18L-induced increase in membrane permeability of PC/PE vesicles. Although Ac-18A-NH_2 inhibited the ability of 18L to cause leakage of PE-containing vesicles, the permeabilizing effects of Ac-18A-NH_2 and 18L on PG-containing vesicles were additive [29]. Therefore, it was of interest to also determine their effects on the fatty acid spin labels in POPE.

Both peptides increased the order of 5-S-SL in POPE (Fig. 5) but 18L had less effect on POPE than on PC/PG vesicles while Ac-18A-NH_2 had a greater effect on POPE than on PC/PG vesicles (compare Figs. 4 and 5). This indicates that the small effect of Ac-18A-NH_2 on 5-S-SL in PC/PG vesicles was not due to low binding of the peptide since it binds much better to PC/PG vesicles than to PE vesicles [37]. 18L also had a small ordering effect on 12-S-SL in POPE but Ac-18A-NH_2 had almost no effect. 18L had a greater effect on 5-S-SL and 12-S-SL than Ac-18A-NH_2 at low P/L ratios but the effects of Ac-18A-NH_2 and 18L were more similar at high P/L ratios (0.05). In contrast to their ordering effects on probes deeper in the bilayer in PC/PG, both peptides disordered or increased the motion of PG-14-S-SL and 16-S-SL. However, 18L increased the motion of 16-S-SL in POPE at all P/L ratios, but Ac-18A-NH_2 had a biphasic effect; it increased the motion at low P/L ratios but had less effect at high P/L ratios. Since the motion of 16-S-SL was not sufficiently isotropic at 31°C to measure τ_o, h_0/h_{+1} is shown instead as it is also sensitive to changes in the frequency and amplitude of motion. For comparison of the relative effect, the change in

both τ_o and h_0/h_{+1} for 16-S-SL in PC/PG are shown in Fig. 4. The magnitude of the effect on h_0/h_{+1} is greater and in the opposite direction for PE compared to PC/PG.

3.4. Induction of lipid gel phase interdigitation by Ala_{19}-Mag and 18L

At high P/L ratios, another antibiotic, polymyxin B, as well as a number of surface active compounds such as ethanol can induce interdigitation of the lipid chains in the gel phase [40,41]. This is related to the ability of these compounds to partition into the apolar/polar interface region and laterally separate the lipid molecules. Compounds such as this should perturb lipid chain order in the liquid crystalline phase where interdigitation cannot occur. Therefore, as an additional measure of the ability of the peptides to partition into the interface region, the ability of Ala_{19}-Mag, 18L, and Ac-18A-NH_2 to induce interdigitation of DPPG at the P/L ratio of 0.2 was determined using 16-S-SL. At this high P/L ratio, the number of positively charged residues (four for 18L and Ac-18A-NH_2 and 5–6 for Ala_{19}-Mag, depending on whether the His is protonated) is sufficient or nearly sufficient to saturate all the negatively charged lipid so that all of the lipid will be affected identically. We have shown that 16-S-SL can be used to detect interdigitated lipid bilayers since the nitroxide group is located near the apolar/polar interface in such bilayers and is much more motionally restricted than in non-interdigitated bilayers where it localizes at the bilayer center [35,42,43]. Indeed, both Ala_{19}-Mag and 18L caused motional restriction of 16-S-SL in the gel phase of DPPG. The T_{max} value was increased from 25.6 G for the pure lipid to about 30 G by both peptides at 7°C indicating that Ala_{19}-Mag and 18L both cause interdigitation of DPPG in the gel phase. The fraction of the probe which was motionally restricted decreased with increasing temperature but motional restriction for a fraction of the probe was maintained on heating up to 27°C for both peptides. Above this temperature, an isotropic component was observed indicating that some of the lipid had gone into the liquid crystalline phase. Ac-18A-NH_2 caused a decrease in T_{max} of 16-S-SL to 21.3 G at 7°C indicating that this peptide did not induce interdigitation of DPPG, but rather had a disordering effect on the lipid gel phase. In the liquid crystalline phase of the lipid with all three peptides, the spectrum was characteristic of rapid isotropic motion as occurs in the pure lipid but the motional parameter was increased by 0.40 nsec for Ala_{19}-Mag, 0.34 ns for 18L, and 0.14 ns for Ac-18A-NH_2, consistent with the relative differences in effects of these peptides on 16-S-SL in PC/PG vesicles. No compo-

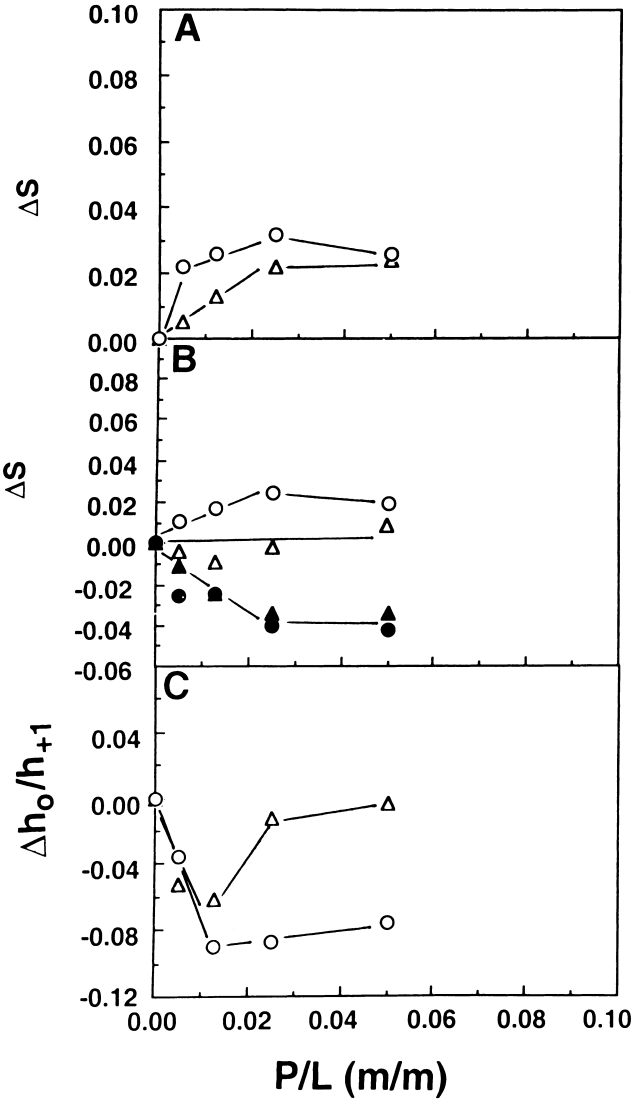

Fig. 5. Effect of Ac-18A-NH_2 and 18L on EPR parameters of spin labels in POPE vesicles at 31°C. Change in parameters with increase in P/L ratio for (A) ΔS of 5-S-SL for Ac-18A-NH_2 (△) and 18L (○); (B) ΔS of 12-S-SL for Ac-18A-NH_2 (△) and 18L (○) and PG-14-S-SL for Ac-18A-NH_2 (▲) and 18L (●); and (C) $\Delta h_0/h_{+1}$ of 16-S-SL for Ac-18A-NH_2 (△) and 18L (○). The accuracy of measurement of the changes is ±0.01 for ΔS and ±0.01 for $\Delta h_0/h_{+1}$.

nent characteristic of more restricted motion was present in the liquid crystalline phase with any of the peptides.

3.5. *Effect of peptides on the gel to liquid crystalline phase transition temperature of DPPG*

The effect of Ala_{19}-Mag, 18L, and Ac-18A-NH_2 on the lipid gel to liquid crystalline phase transition temperature of DPPG was monitored from changes in the peak height of the spectrum, since this reflects changes in mobility of the spin label. At a P/L ratio of 0.2, Ala_{19}-Mag decreased the phase transition temperature by about 8°C (Fig. 6A) and 18L by about 4–5°C (Fig. 6B). There was significant hysteresis in the change in spectra of 16-S-SL in the lipid-peptide samples on cooling compared to heating, particularly for Ala_{19}-Mag. A plot of the peak height with temperature on cooling indicated that Ala_{19}-Mag decreased the liquid crystalline to gel phase transition temperature by 12°C, and 18L decreased it by 8°C (Fig. 6A,B). The spectra remained as sharp three-line spectra, characteristic of rapid isotropic motion, until 28°C where the spin label again became motionally restricted. The heating and cooling curves for the pure lipid sample were nearly identical to each other. Ala_{19}-Mag was reported earlier to decrease the phase transition temperature of DPPG by 3–4°C at a P/L mole ratio of 0.09, as detected by FTIR spectroscopy [17]. At lower P/L ratios no significant effect on the phase transition could be detected using FTIR spectroscopy (Blazyk, unpublished). Ac-18A-NH_2 decreased the gel to liquid crystalline phase transition temperature of DPPG by about 10°C and the transition was significantly broader than with the other peptides (Fig. 6C). There was no hysteresis between heating and cooling curves.

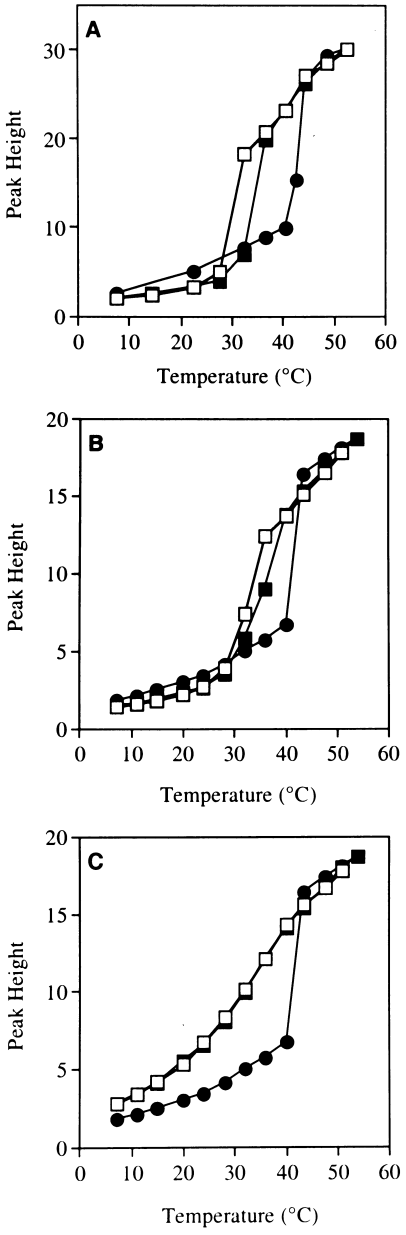

Fig. 6. Effect of (A) Ala_{19}-Mag, (B) 18L, and (C) Ac-18A-NH_2 on the gel to liquid crystalline phase transition temperature of DPPG on heating and cooling, measured from the change in height of the center peak (in arbitrary units) of the EPR spectrum of 16-S-SL with temperature. DPPG alone (●) and DPPG-peptide, heating (■) and cooling (□). The height for the lipid-peptide samples was adjusted so that it coincided with the height for the lipid sample at the highest temperature. Data points on heating and cooling curves which cannot be distinguished coincided with each other. Curves for the lipid only and the Ac-18A-NH_2-lipid sample were identical on heating and cooling.

4. Discussion

The properties of these peptides and effects on lipid behavior shown here and reported elsewhere are compared qualitatively in Table 1. The effect of the magainin and 18L peptides on fatty acid spin labels in liquid crystalline phase PC/PG bilayers indicates that they significantly increased lipid chain

order near the apolar/polar interface region and also had small ordering or motional restricting effects on the portions of the lipid chains deeper in the bilayer, at carbons 12–16. The effects of the magainins, especially that of Mag, were somewhat smaller than those of 18L. Ac-18A-NH_2 on the other hand had much smaller effects. This ordering effect of 18L and magainins, particularly near the apolar/polar interface, is indicative of penetration into this region by hydrophobic amino acid side chains from the hydrophobic side of the amphipathic helices lying on the surface of the bilayer. The order of effect on chain order in PC/PG bilayers, 18L > Ala_{19}-Mag > Mag > Ac-18A-NH_2, correlates with the width of the hydrophobic face of these peptides and with the number of hydrophobic amino acids with bulky side chains (Table 1). This supports a decrease in penetration of the peptide hydrophobic face into the apolar/polar interface in the same order. It has recently been shown for the basic domain of the MARCKS protein that large hydrophobic amino acids such as Phe cause deeper penetration of the peptide into the bilayer interface by 10–15 Å relative to a similar peptide containing Phe to Ala substitutions [44]. The small effect of Ac-18A-NH_2 on the lipid acyl chains is consistent with an X-ray diffraction study in which Ac-18A-NH_2 was found located close to the glycerol moiety of DOPC, causing only a slight decrease in bilayer thickness due to its small perturbing effect [45].

Some degree of penetration of hydrophobic side chains of magainins and 18L from the peptide helices on the bilayer surface is also indicated by the ability of Ala_{19}-Mag and 18L, at a high P/L ratio, to cause interdigitation of the lipid chains of DPPG in the gel phase. This is shown by the significant restriction of the motion of 16-S-SL in the gel phase by these peptides. Similar motional restriction of 16-S-SL is caused by a number of compounds which have been shown by X-ray diffraction to induce interdigitation of gel phase lipids, such as polymyxin B and amphipathic small molecules such as ethanol, glycerol [35,43], and KSCN [46]. These compounds are located in the apolar/polar interface of the gel phase with their hydrophobic side chains or surfaces dipping into the hydrophobic region of the bilayer thus causing lateral separation of the lipid molecules. The lipid chains from the other side of the bilayer then interdigitate into the space produced. Interdigitation results in location of the nitroxide group on 16-S-SL near the apolar/polar interface, a less fluid region of the bilayer than the bilayer center. Thus the motional restriction of 16-S-SL induced by Ala_{19}-Mag and 18L indicate that they are also able to induce interdigitation of DPPG in the gel phase. We reached a similar conclusion for myelin basic protein, which is also thought to bind to the bilayer surface with hydrophobic side chains dipping into the bilayer [42,47].

The decrease in transition temperature caused by Ala_{19}-Mag and 18L indicates that in spite of inducing interdigitation of the lipid chains in the gel phase, these peptides preferentially interact with and stabilize the liquid crystalline phase. This indicates greater insertion of the peptide into the liquid crystalline phase bilayer while lying parallel to the bilayer surface. This contrasts with several other substances which induce interdigitation, such as ethanol, glycer-

Table 1

Result	Order of effect	Reference
Angle subtended by hydrophobic face[a]	18L > Ala_{19}-Mag > Mag = Ac-18A-NH_2	[27,30]
Number of bulky hydrophobic residues[b]	18L > magainins > Ac-18A-NH_2	
Permeabilization of PG-containing vesicles	Ala_{19}-Mag > Mag > Ac-18A-NH_2 ≫ 18L	this study
Permeabilization of PE-containing vesicles	18L increase, Ac-18A-NH_2 inhibits increase induced by 18L	[27,29]
T_H of PE	18L decrease (negative curvature)	[27]
	Ac-18A-NH_2 increase (positive curvature)	[27]
	Mag increase (positive curvature)	[24]
Acyl chain ordering of PC/PG	18L > Ala_{19}-Mag > Mag > Ac-18A-NH_2	this study
16-S-SL disordering of PE	18L > Ac-18A-NH_2	this study

[a]The angle reported by Dathe et al. [30] is for the angle subtended by the basic residues. Mag also has an acidic residue at position 19 which decreases the size of its hydrophobic face compared to 18L and Ala_{19}-Mag.
[b]Leu, Ile, Trp, Phe.

ol, polymyxin B, and myelin basic protein, which increase or only slightly decrease the phase transition temperature of DPPC or DPPG on heating [42,43,48]. However, the large hysteretic effect of Ala_{19}-Mag and 18L in DPPG on cooling resembles that observed with myelin basic protein and glycerol in DPPG. Polymyxin B and ethanol had much smaller hysteretic effects on lipids [35,43,49]. The hysteresis indicates a kinetic barrier to reformation of the interdigitated gel phase on cooling. The greater mobility of 16-S-SL caused by Ac-18A-NH_2 indicates that it does not induce interdigitation of DPPG. Its inability to induce interdigitation may reflect its lower degree of penetration into the bilayer interface or may indicate a greater kinetic barrier to interdigitation in the presence of this peptide. It significantly decreases the phase transition temperature indicating a large perturbing effect on the gel phase at a P/L mole ratio of 0.2. Ac-18A-NH_2 caused clearing of the DPPG suspension and electron microscopy showed that this was due to fragmentation of the vesicles. The unblocked form of the peptide, 18A, caused fragmentation of DMPC bilayers into discs [50] but did not have this effect on PC with an unsaturated fatty acid [51]. However, the blocked form, Ac-18A-NH_2, caused fragmentation of both DMPC and unsaturated PC into discs [31]. Its inability to induce interdigitation would result in an increase in positive intrinsic monolayer curvature and destabilization of large vesicles, and thus lead to disc formation.

Only a few other studies have reported effects of magainin related to lipid chain order. Magainin caused a decrease in thickness of PC/PS bilayers of about 2 Å at a P/L ratio of 0.015 indicating a small perturbation of lipid chain order [52]. Raman spectroscopy showed that at a P/L mole ratio of 0.33, it increased the *gauche*/*trans* isomerization of the acyl chains of DPPG [53]; lower P/L ratios were not investigated. At high P/L ratios, Mag also disordered the fatty acyl chains of lipopolysaccharides detected by FTIR spectroscopy [54]. On the other hand, a ^{2}H-NMR study indicated that Mag at a P/L ratio of 0.05 had little effect on the order of the double bond of deuterated PC in PC/PG 3:1 vesicles [55]. Matsuzaki et al. [56] found that the phase transition temperature of DPPG was increased slightly, rather than decreased, at a P/L ratio of 0.036. However, Hirsh et al. [17] showed that at a high P/L ratio, Ala_{19}-Mag decreased the phase transition temperature of DPPG, consistent with the results presented here. Some of these discrepancies may be due to the different lipids, type of magainin, or different conditions used.

The most important result from this study is that there was no correlation between the effects of the three peptide classes on lipid chain order in PC/PG and their lytic effects. Ac-18A-NH_2 caused much more leakage than 18L, almost as much as the magainins, even though Ac-18A-NH_2 had much less effect on lipid chain order than 18L and the magainins. Since the degree of perturbation of the lipid chains caused by 18L at a P/L ratio of 0.05 is not sufficient to significantly increase permeability, this indicates that the similar perturbing effect of the magainins is not responsible for their permeabilization of vesicles. Thus magainins probably increase permeability of PG-containing vesicles by forming some type of pore as indicated by other evidence [7–12,14,18–21]. 18L, which does not form stable pores, at least in PC [29], is not able to cause much leakage at P/L ratios of 0.05. However, the large amount of leakage which 18L causes at higher P/L ratios could be due to its effects on lipid chain order. Higher P/L ratios for magainin are required to cause leakage of vesicles containing PS, PA, and CL than those containing PG and the absence of a cooperative effect on the former vesicles suggests that magainin does not form pores in those vesicles. Its larger perturbing effect on lipid chain order at high P/L ratios may then be responsible for the leakage which it causes in vesicles containing PS, PA, and CL.

Ac-18A-NH_2, however, causes leakage of PG-containing vesicles at similar P/L ratios as magainins while having little effect on lipid chain order. Possibly Ac-18A-NH_2 also forms pores in PG-containing vesicles, although no evidence for this has yet been reported. It does not form pores in PC bilayers [29]. More likely, its lytic effect on PG-containing vesicles is due to fragmentation of the vesicles into discs or other small membrane fragments as found here for DPPG, and earlier for DMPC and unsaturated PCs [31]. We observed that Ac-18A-NH_2 also inhibited sedimentation of PC/PG MLVs relative to 18L although the structure of the vesicles was not investigated by electron microscopy.

In vesicles containing PE, the abilities of 18L to cause leakage and of Ac-18A-NH_2 to inhibit leakage have been shown to correlate with their effects on T_H and on intrinsic lipid monolayer curvature [27,29]. In contrast to its lipid chain ordering effect in PG-containing bilayers, 18L increased the chain motion at carbons 12 and 16 in POPE. This is consistent with an increase in negative curvature of this lipid, indicated by the fact that 18L decreases T_H of PE [57]. Using these spin labels, we showed earlier that the lipid acyl chains have a greater amplitude of motion, particularly near the chain ends, in the hexagonal phase of PE than in the lamellar phase [58]. Ac-18A-NH_2, which increases T_H of PE, indicating that it promotes positive curvature of PE, had a disordering effect on 16-S-SL only at low P/L ratios. Thus the effects of these peptides on lipid chain order in PE are consistent with their effects on T_H and indicate that 18L promotes negative curvature while Ac-18A-NH_2 inhibits it, particularly at high P/L ratios. This would result in increased leakage of PE-containing vesicles by 18L and inhibition of leakage of PE-containing vesicles by Ac-18A-NH_2. Thus the mechanisms of vesicle permeabilization by these peptides depend on both the peptide and on the lipid composition of the vesicles.

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References

[1] M.G. Giovannini, L. Poulter, B.W. Gibson, D.H. Williams, Biosynthesis and degradation of peptides from *Xenopus laevis* prohormones, Biochem. J. 243 (1987) 113–120.
[2] M. Zasloff, B. Martin, H.-C. Chen, Antimicrobial activity of synthetic magainin peptides and several analogues, Proc. Natl. Acad. Sci. USA 85 (1988) 910–913.
[3] H.-C. Chen, J.H. Brown, J.L. Morell, C.M. Huang, Synthetic magainin analogues with improved antimicrobial activity, FEBS Lett. 236 (1988) 462–466.
[4] M.A. Baker, W.L. Maloy, M. Zasloff, L.S. Jacob, Anticancer efficacy of magainin 2 and analogue peptides, Cancer Res. 53 (1993) 3052–3057.
[5] K. Matsuzaki, M. Harada, T. Handa, S. Funakoshi, N. Fujii, H. Yajima, K. Miyajima, Magainin 1-induced leakage of entrapped calcein out of negatively charged lipid vesicles, Biochim. Biophys. Acta 981 (1989) 130–134.
[6] E. Grant Jr., T.J. Beeler, K.M.P. Taylor, K. Gable, M.A. Roseman, Mechanism of magainin 2a-induced permeabilization of phospholipid vesicles, Biochemistry 31 (1992) 9912–9918.
[7] R.A. Cruciani, J.L. Barker, M. Zasloff, H.-C. Chem, O. Colamonici, Antibiotic magainins exert cytolytic activity against transformed cell lines through channel formation, Proc. Natl. Acad. Sci. USA 88 (1991) 3792–3796.
[8] R.A. Cruciani, J.L. Barker, S.R. Durell, G. Raghunathan, H.R. Guy, M. Zasloff, E.F. Stanley, Magainin 2, a natural antibiotic from frog skin, forms ion channels in lipid bilayer membranes, Eur. J. Pharmacol. 226 (1992) 287–296.
[9] B. Haimovich, J.C. Tanaka, Magainin-induced cytotoxicity in eukaryotic cells: kinetics, dose response, and channel characteristics, Biochim. Biophys. Acta 1240 (1995) 149–158.
[10] H. Duclohier, G. Molle, G. Spach, Antimicrobial peptide magainin 1 from Xenopus skin forms anion-permeable channels in planar lipid bilayers, Biophys. J. 56 (1989) 1017–1021.
[11] K. Matsuzaki, O. Murase, H. Tokuda, S. Funakoshi, N. Fujii, K. Miyajima, Orientational and aggregational states of magainin 2 in phospholipid bilayers, Biochemistry 33 (1994) 3342–3349.
[12] K. Matsuzaki, O. Murase, N. Fujii, K. Miyajima, Translocation of a channel-forming antimicrobial peptide, magainin 2, across lipid bilayers by forming a pore, Biochemistry 34 (1995) 6521–6526.
[13] S.J. Ludtke, K. He, Y. Wu, H.W. Huang, Cooperative membrane insertion of magainin correlated with its cytolytic activity, Biochim. Biophys. Acta 1190 (1994) 181–184.
[14] S.J. Ludtke, K. He, W.T. Heller, T.A. Harroun, L. Yang, H.W. Huang, Membrane pores induced by magainin, Biochemistry 35 (1996) 13723–13728.
[15] B. Bechinger, M. Zasloff, S.J. Opella, Structure and interactions of magainin antibiotic peptides in lipid bilayers. A solid state nuclear magnetic resonance investigation, Biophys. J. 62 (1992) 12–14.
[16] B. Bechinger, M. Zasloff, S.J. Opella, Structure and orientation of the antibiotic peptide magainin in membranes by solid-state nuclear magnetic resonance spectroscopy, Protein Sci. 2 (1993) 2077–2084.
[17] D.J. Hirsh, J. Hammer, W.L. Maloy, J. Blazyk, J. Schaefer, Secondary structure and location of a magainin analogue in synthetic phospholipid bilayers, Biochemistry 35 (1996) 12733–12741.
[18] K. Matsuzaki, O. Murase, K. Miyajima, Kinetics of pore formation by an antimicrobial peptide, magainin 2, in phospholipid bilayers, Biochemistry 34 (1995) 12553–12559.

[19] K. Matsuzaki, O. Murase, N. Fujii, K. Miyayama, An antimicrobial peptide, magainin 2, induced rapid flip flop of phospholipids coupled with pore formation and peptide translocation, Biochemistry 35 (1996) 11361–11368.
[20] E. Jo, J. Blazyk, J.M. Boggs, Insertion of magainin into the lipid bilayer detected using lipid photolabels, Biochemistry 37 (1998) 13791–13799.
[21] M.R. Wenk, J. Seelig, Magainin 2 amide interaction with lipid membranes: calorimetric detection of peptide binding and pore formation, Biochemistry 37 (1998) 3909–3916.
[22] B. Bechinger, The structure, dynamics and orientation of antimicrobial peptides in membranes by multidimensional solid-state NMR spectroscopy, Biochim. Biophys. Acta 1462 (1999) 157–183.
[23] S. Berneche, M. Nina, B. Roux, Molecular dynamics simulation of melittin in a dimyristoylphosphatidylcholine bilayer membrane, Biophys. J. 75 (1998) 1603–1618.
[24] K. Matsuzaki, K.-I. Sugishita, N. Ishibe, M. Ueha, S. Nakata, K. Miyajima, R.M. Epand, Relationship of membrane curvature to the formation of pores by magainin 2, Biochemistry 37 (1998) 11856–11863.
[25] T. Wieprecht, M. Beyermann, J. Seelig, Binding of antibacterial magainin peptides to electrically neutral membranes: thermodynamics and structure, Biochemistry 38 (1999) 10377–10387.
[26] J.H. Cuervo, B. Rodriguez, R.A. Houghten, in: J.E. Rivier, G.R. Marshall (Eds.), Peptides: Chemistry and Biology, ESCOM, Leiden, 1990, pp. 124–126.
[27] E.M. Tytler, J.P. Segrest, R.M. Epand, S.Q. Nie, R.F. Epand, V.K. Mishra, Y.V. Venkatachalapathi, G.M. Anantharamaiah, Reciprocal effects of apolipoprotein and lytic peptide analogs on membranes, J. Biol. Chem. 268 (1993) 22112–22118.
[28] J.P. Segrest, H. De Loof, J.G. Dohlman, C.G. Brouilette, G.M. Anantharamaiah, Amphipathic helix motif: classes and properties, Proteins 8 (1990) 103–117.
[29] I.V. Polozov, A.I. Polozova, E.M. Tytler, G.M. Anantharamaiah, J.P. Segrest, G.A. Wooley, R.M. Epand, Role of lipids in the permeabilization of membranes by class L amphipathic helical peptides, Biochemistry 36 (1997) 9237–9245.
[30] M. Dathe, T. Wieprecht, H. Nikolenko, L. Handel, W.L. Maloy, D.L. MacDonald, M. Beyermann, M. Bienert, Hydrophobicity, hydrophobic moment and angle subtended by charged residues modulate antibacterial and haemolytic activity of amphipathic helical peptides, FEBS Lett. 403 (1997) 208–212.
[31] V.V. Venkatachalapathi, M.C. Phillips, R.M. Epand, R.F. Epand, E.M. Tytler, J.P. Segrest, G.M. Anantharamaiah, Effect of end group blockage on the properties of a class A amphipathic helical peptide, Proteins 15 (1993) 349–359.
[32] B.H. Chung, M.N. Palgunachari, V.K. Mishra, C.H. Chang, J.P. Segrest, G.M. Anantharamaiah, Probing structure and function of VLDL by synthetic amphipathic helical peptides, J. Lipid Res. 37 (1996) 1099–1112.
[33] G.M. Anantharamaiah, Synthetic peptide analogs of apolipoproteins, Methods Enzymol. 128 (1986) 627–647.
[34] J.M. Boggs, M.A. Moscarello, Effects of basic protein from human CNS myelin on lipid bilayer structure, J. Membr. Biol. 39 (1978) 75–96.
[35] J.M. Boggs, G. Rangaraj, A. Watts, Behavior of spin labels in a variety of interdigitated lipid bilayers, Biochim. Biophys. Acta 981 (1989) 243–253.
[36] H. Ellens, J. Bentz, F.C. Szoka, pH-induced destabilization of phosphatidylethanolamine-containing liposomes: role of bilayer contact, Biochemistry 23 (1984) 1532–1538.
[37] I.V. Polozov, A.I. Polozova, V.K. Mishra, G.M. Anantharamaiah, J.P. Segrest, R.M. Epand, Studies of kinetics and equilibrium membrane binding of class A and class L model amphipathic peptides, Biochim. Biophys. Acta 1368 (1998) 343–354.
[38] K. Matsuzaki, M. Harada, S. Funakoshi, N. Fujii, K. Miyajima, Physicochemical determinants for the interactions of magainins 1 and 2 with acidic lipid bilayers, Biochim. Biophys. Acta 1063 (1991) 162–170.
[39] P.C. Jost, O.H. Griffith, R.A. Capaldi, G. Vanderkooi, Evidence for boundary lipid in membranes, Proc. Natl. Acad. Sci. USA 70 (1973) 480–484.
[40] J.-L. Ranck, J.-F. Tocanne, Polymyxin B induces interdigitation in dipalmitoylphosphatidylglycerol lamellar phase with stiff hydrocarbon chains, FEBS Lett. 143 (1982) 175–178.
[41] S.A. Simon, T.J. McIntosh, Interdigitated hydrocarbon chain packing causes the biphasic transition behavior in lipid/alcohol suspensions, Biochim. Biophys. Acta 773 (1984) 169–172.
[42] J.M. Boggs, D. Stamp, M.A. Moscarello, Interaction of myelin basic protein with dipalmitoylphosphatidylglycerol: dependence on the lipid phase and investigation of a metastable state, Biochemistry 20 (1981) 6066–6072.
[43] J.M. Boggs, G. Rangaraj, Phase transitions and fatty acid spin label behavior in interdigitated lipid phases induced by glycerol and polymixin, Biochim. Biophys. Acta 816 (1985) 221–233.
[44] K. Victor, J. Jacob, D.S. Cafiso, Interactions controlling the membrane binding of basic protein domains: phenylalanine and the attachment of the myristoylated alanine-rich C-kinase substrate protein to interfaces, Biochemistry 38 (1999) 12527–12536.
[45] K. Hristova, W.C. Wimley, V.K. Mishra, G.M. Anantharamiah, J.P. Segrest, S.H. White, An amphipathic α-helix at a membrane interface: a structural study using a novel X-ray diffraction method, J. Mol. Biol. 290 (1999) 99–117.
[46] R. Bartucci, S. Belsito, L. Sportelli, Neutral lipid bilayers interacting with chaotropic anions, Chem. Phys. Lipids 79 (1996) 171–180.
[47] J.M. Boggs, D.D. Wood, M.A. Moscarello, Hydrophobic and electrostatic interactions of myelin basic protein with lipid. Participation of N-terminal and C-terminal portions, Biochemistry 20 (1981) 1065–1073.
[48] E.S. Rowe, Lipid chain length and temperature dependence

of ethanol-phosphatidylcholine interactions, Biochemistry 22 (1983) 3299–3305.
[49] E.S. Rowe, Thermodynamic reversibility of phase transitions. Specific effects of alcohols on phosphatidylcholines, Biochim. Biophys. Acta 813 (1985) 321–330.
[50] G.M. Anantharamaiah, J.I. Jones, C.G. Brouillette, C.F. Schmidt, B.H. Chung, T.A. Hughes, A.S. Bhown, J.P. Segrest, Studies of synthetic peptide analogs of the amphipathic helix, J. Biol. Chem. 260 (1985) 10248–10255.
[51] P. Spuhler, G.M. Anantharamaiah, J.P. Segrest, J. Seelig, Binding of apolipoprotein A-I model peptides to lipid bilayers, J. Biol. Chem. 269 (1994) 23904–23910.
[52] S. Ludtke, K. He, H. Huang, Membrane thinning caused by magainin 2, Biochemistry 34 (1995) 16764–16769.
[53] R.W. Williams, R. Starman, K.M.P. Taylor, K. Gable, T. Beeler, M. Zasloff, D. Covell, Raman spectroscopy of synthetic antimicrobial frog peptides, magainins 2a and PGLa, Biochemistry 29 (1990) 4490–4496.
[54] F.R. Rana, E.A. Macias, C.M. Sultany, M.C. Modzrakowski, J. Blazyk, Interactions between magainin 2 and *Salmonella typhimurium* outer membranes: effect of lipopolysaccharide structure, Biochemistry 30 (1991) 5858–5866.
[55] K. Matsuzaki, J. Seelig, NMR study on interactions of an antibiotic peptide, magainin 2, with lipid bilayers, in: M. Ohno (Ed.), Peptide Chemistry, Protein Research Foundation, Osaka, 1994, pp. 129–132.
[56] K. Matsuzaki, M. Fukui, N. Fujii, K. Miyajima, Interactions of an antimicrobial peptide, tachyplesin I, with lipid membranes, Biochim. Biophys. Acta 1070 (1991) 259–264.
[57] T. Wieprecht, M. Dathe, R.M. Epand, M. Beyermann, E. Krause, W.L. Maloy, D.L. MacDonald, M. Biener, Influence of the angle subtended by the positively charged helix face on the membrane activity of amphipathic, antibacterial peptides, Biochemistry 36 (1997) 12869–12880.
[58] J.M. Boggs, D. Stamp, D.W. Hughes, C.M. Deber, Influence of the ether linkage on the lamellar to hexagonal phase transition of ethanolamine phospholipids, Biochemistry 20 (1981) 5728–5735.