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Lipid interactions of LAH4, a peptide with antimicrobial and nucleic acid transfection activities

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Abstract The cationic amphipathic designer peptide LAH4 exhibits potent antimicrobial, nucleic acid transfection and cell penetration activities. Closely related derivatives have been developed to enhance viral transduction for gene therapeutic assays. LAH4 contains four histidines and, consequently, its overall charge and membrane topology in lipid bilayers are strongly pH dependent. In order to better understand the differential interactions of this amphipathic peptide with negatively-charged membranes its interactions, topologies, and penetration depth were investigated in the presence of lipid bilayers as a function of pH, buffer, phospholipid head group, and fatty acyl chain composition using a combination of oriented synchrotron radiation circular dichroism spectroscopy as well as oriented and non-oriented solid-state NMR spectroscopy. This combination of methods indicates that in the presence of lipids with phosphatidylglycerol head groups, the topological equilibria of LAH4 is shifted towards more in-plane configurations even at neutral pH. In contrast, a transmembrane alignment is promoted when LAH4 interacts with membranes made of dimyristoyl phospholipids rather than palmitoyl-oleoyl-phospholipids. Finally, the addition of citrate buffer favours LAH4 transmembrane alignments, even at low pH, probably by complex formation with the cationic charges of the peptide. In summary, this study has revealed that the membrane topology of this peptide is readily modulated by the environmental conditions.

Keywords Peptide-membrane interactions · Membrane topology · Oriented solid-state NMR Spectroscopy · Oriented synchrotron radiation circular dichroism (oSRCD) spectroscopy · Transmembrane helix · In-plane alignment

Abbreviations

CD	Circular dichroism
DMPC	1, 2-Dimyristoyl-*sn*-glycero-3-phosphocholine
DMPG	1, 2-Dimyristoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol)
DSPC	1, 2-Distearoyl-*sn*-glycero-3-phosphocholine
DPPC	1, 2-Dipalmitoyl-*sn*-glycero-3-phosphocholine
hΦ17, KALP	GKKLA LALAL ALALA LALAL KKA-$CONH_2$
KL14	KKLLK KAKKL LKKL-$CONH_2$
LAH4	KKALL ALALH HLAHL ALHLA LALKK A-$CONH_2$
NMR	Nuclear magnetic resonance
oSRCD	Oriented synchrotron radiation circular dichroism
PC	Phosphatidylcholine
PG	Phosphatidylglycerol
PGLa	GMASK AGAIA GKIAK VALKA L-$CONH_2$
POPC	1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine
POPG	1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol)

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r.h.	Relative humidity
SAXS	Small angle X-ray scattering
SRCD	Synchrotron radiation circular dichroism

Introduction

When antimicrobial peptides such as magainin and cecropin were discovered, their mode of action was soon associated with their possibility to interact with bacterial membranes and the formation of pores (Zasloff 2002). Biophysical methods have revealed that, while some more hydrophobic peptide sequences do traverse the membrane (Sansom 1993; Woolley and Wallace 1992), these highly cationic sequences preferred to align parallel to the membrane surface (Bechinger 1999, 2011) suggesting the evolution of two separate mechanisms for peptide antimicrobials.

The synthetic LAH4 peptides were designed specifically to investigate the interactions that determine the membrane topology of helical peptides (Bechinger 1996). Their sequences, which are rich in alanine and leucine residues, are compatible with a transmembrane environment, while the two lysines situated at each terminus assure good solubility in water. The LAH4 sequence includes four histidines with pK_a values between 5.4 and 6.0 when measured in dodecylphophocholine micellar environments (Bechinger 1996; Georgescu et al. 2010). These are spaced so that, when the peptide adopts a helical structure, they line up on one surface and allow the hydrophobic moment of the peptide to be tuned as a function of pH. This, in turn, affects the membrane alignment of the peptide, which adopts a transmembrane configuration in neutral conditions and an in-plane orientation at pH <5.5 (Aisenbrey et al. 2006a, b; Bechinger 1996).

Members of this family of peptides exhibit membrane pore-formation (Marquette et al. 2008) and antimicrobial action at both neutral and at acidic pH (Vogt and Bechinger 1999), including against clinical isolates where the low pH configuration is more active (Mason et al. 2006a, 2009). More recently peptides of the LAH4 family have been found to also exhibit potent DNA and siRNA transfection activities (Kichler et al. 2003; Langlet-Bertin et al. 2010; Mason et al. 2006a). LAH4 can therefore act as a non-viral vector and the peptide has indeed been used to enable intracellular delivery of quantum dots (Gemmill et al. 2013), or of protein-based vaccines adjuvanted with CpG oligonucleotide in order to generate enhanced immune responses and antitumor effects (Zhang et al. 2011). Furthermore, transduction by adeno-associated viruses is enhanced by LAH4 (Liu et al. 2014), and an LAH4 derivative has been developed, which greatly enhances lentiviral transduction in cell culture, methods that are used in the clinic for gene therapeutic approaches (Fenard et al. 2013).

Membrane interactions of LAH4 are important to explain both the antimicrobial as well as the cell penetrating peptide activities. The formation of pores in bacterial or fungal membranes is considered a key event for the development of antimicrobial activities of many amphipathic peptides (Zasloff 2002), whilst during transfection experiments extended LAH4 peptide/nucleic acid complexes enter the cell via an endosomal pathway (Kichler et al. 2003). Upon acidification of this organelle, the liberation of a large fraction of membrane-active peptides assures endosomal release of the genetic information into the cytoplasm (Prongidi-Fix et al. 2007b).

Interestingly, for LAH4 (Vogt and Bechinger 1999) and other peptides (Cheng et al. 2011; Mason et al. 2006b), differences have been observed in activity when membranes containing different amounts of palmitoyl-oleoyl-phosphatidylglycerol (POPG) are compared. The latter is of interest as bacteria expose a considerable amount of phosphatidylglycerol (PG) at the outer membrane layer, and acidic lipids are an integral part of the internal organelles of eukaryotes. Whereas a number of studies have been performed on the LAH4 interactions with zwitterionic lipids (Bechinger 1996), the alignment of these peptides in bilayers made of negatively charged lipids has never been reported.

Here, we present studies of the topology of LAH4 in PG membranes using solid-state NMR, oriented CD and oriented synchrotron radiation circular dichroism (oSRCD) spectroscopies. The oSRCD has the advantage over oriented CD spectroscopy using conventional CD instruments, in that it enables measurements to lower wavelengths providing a more accurate measure of orientation and secondary structure. As a control we included two model sequences with stable in-plane and transmembrane orientations. The combination of these approaches allowed us to obtain a comprehensive view of the LAH4 topology and correspondingly to better explore the functional differences between anionic POPG and zwitterionic POPC membranes. Much of our previous work on members of the LAH4 peptide family has been performed with palmitoyl-oleoyl-phospholipids, which represent well the fatty acyl chain composition of most biological membranes. However dimyristoyl-lipids have been used for the study of other peptides or for experimental approaches including neutron diffraction techniques, and interesting differences have been detected (Salnikov and Bechinger 2011; Strandberg et al. 2013). Therefore, this work encompassed also a comparison of the interactions of LAH4 not only with membranes made of different head groups but also as a function of fatty acyl chain composition. Furthermore, the peptide topology was found to be dependent on the presence of citrate buffer, which added another, so far unexplored, variable influencing peptide topologies.

Materials and methods

Peptide synthesis and chemicals

Two isotopologues of the peptide LAH4 (KKALL ALA LH HLAHL ALHLA LALKK A-$CONH_2$) as well as the peptides KALP/hΦ17 (GKKLA LALAL ALALA LALAL KKA-$CONH_2$) and KL14 (KKLLK KAKKL LKKL-$CONH_2$) were prepared by automated solid-phase synthesis using standard Fmoc chemistry on a Millipore 9050 synthesizer. A single ^{15}N-labelled alanine was incorporated at position 16 for [^{15}N-Ala_{16}]-LAH4, while the [^{15}N-$Ala_{6,16,22}$]-LAH4 analogue was synthesized with three ^{15}N-labeled alanines at positions 6, 16 and 22. The purity and identity of the products was assessed by MALDI-TOF mass spectrometry and HPLC. All the lipids (DMPC, DMPG, POPC, POPG) were from Avanti Polar Lipids Inc. (Alabaster, AL, USA) and used without further purification. Chloroform, methanol, and trifluoroethanol (TFE) were from Sigma Aldrich (Lyon, France).

Sample preparation for solid-state NMR spectroscopy

The samples were prepared following previously described protocols using two different approaches (Aisenbrey et al. 2013; Bechinger et al. 2010): Peptide was dissolved at 8 mg/ml either in 10 mM citrate buffer, pH 4.8 or alternatively in ~500 µL milliQ water. In the latter case the pH was adjusted by the addition of microliter amounts of 1 N NaOH or HCl. The peptide solution was added to 20–25 mg of lipid, vortexed, bath-sonicated, and exposed to three freeze/thaw cycles. The suspension was then dried onto 20–25 glass plates and equilibrated at 93 % relative humidity at ~310 K for two days. The pH value of the lipid/peptide samples was again tested from a small part of the sample that was mixed with 300 µl milliQ water. After equilibration, the glass plates were stacked on top of each other, wrapped with Teflon tape and sealed in plastic wrapping.

When non-oriented samples were to be prepared, the lipid/peptide mixtures were dried and rehydrated in the test tube, and, after equilibration at 93 % relative humidity at ~310 K, the resulting films were transferred into 4 mm zirconia MAS rotors by mild centrifugation.

Solid-state NMR experiments

The solid-state NMR experiments were performed on Bruker Avance 300 and Bruker Avance 400 spectrometers, equipped with static solid-state NMR probes either with a solenoidal coil for non-oriented, or modified with a flattened coil for mechanically oriented samples [shown in (Goldmann et al. 2008)]. Proton-decoupled ^{15}N spectra were acquired with cross polarization using a contact time of 800 µs. Nitrogen and proton fields were swept through the Hartmann-Hahn condition using a tangential time dependence (Hediger et al. 1994). The B_1 fields were in the range 30–35 kHz, the recycle delay was 3 s. Before Fourier-transformation, an exponential apodization function corresponding to a line broadening of 200 Hz was applied.

Sample preparation for CD and SRCD spectroscopy

Approximately 2 mg of each peptide were codissolved with POPC or DMPC (to give peptide-to-lipid ratios of 1:50) in 0.2 ml of TFE (for POPC) or in chloroform/methanol (1:1 v/v) (for DMPC). An aliquot of 20 µl was spread on a circular Suprasil quartz window (Hellma Ltd. UK) and the solvent evaporated using a stream of nitrogen gas. The resulting dry film ~15 mm in diameter was placed under vacuum for at least 2 h to remove residual solvent before being equilibrated overnight in a chamber in which the relative humidity was maintained at ~95 % by a solution of saturated potassium nitrate that was not in direct contact with the films (Wien and Wallace 2005).

Samples containing buffer (10 mM citrate/citric acid pH 5 or sodium phosphate mono acid/citric acid pH 7) were prepared as follows: First the peptide and lipids were codissolved in organic solvents, then the organic solvent was removed as above. The samples were resuspended in buffer (ca. 1.7 mg/30 µl) by vortexing and then incubated for 1 h at 298 K (POPC) or 337 K (DMPC) (this resuspension procedure was carried out twice). 30 µl of suspension were then spread onto a quartz window and dried overnight in air. The dried films obtained were successively equilibrated at 298 K (POPC) or 338 K (DMPC) in a humidity controlled chamber at 95 % relative humidity overnight as described above. For each type of sample, a corresponding baseline sample was prepared in the same manner but without peptide present.

Oriented CD and oSRCD spectroscopy

Spectra were recorded on SRCD beamline UV1 at the ISA facility, University of Aarhus, Denmark. Data was recorded in the wavelength range from 178 to 280 nm in 1 nm steps with 1 nm bandwidth and averaging time of 2 s. In order to assess and minimise the linear dichroism contributions (Vogel 1987), eight spectra were recorded for each sample, after rotating the cell around its central axis in steps of 45 degrees. The eight spectra were averaged, baseline corrected and zeroed between 270 and 280 nm. CD spectra were measured on an AvivD62 spectrophotometer using the same parameters as above except the wavelength range was from 280 to 195 nm.

Results

Solid-state NMR spectroscopy

Previously LAH4 labelled with ^{15}N at selected amide positions was reconstituted into uniaxially oriented POPC membranes and investigated by ^{15}N solid-state NMR spectroscopy. At neutral pH the chemical shifts around 200 ppm are indicative that LAH4 and related peptide helices adopt transmembrane alignments, whereas under acidic conditions, values <100 ppm show that the helices are oriented parallel to the membrane surface (Aisenbrey et al. 2006b; Bechinger 1996). Here, we extended these investigations to POPG membranes at pH 7.4 and at pH 5 (Fig. 1a, b). The ^{15}N solid-state NMR spectra of [^{15}N-Ala$_{16}$]-LAH4 exhibit chemical shift values of 74.5 ± 2.5 ppm (Fig. 1a) and 74.5 ± 3 ppm (Fig. 1b) thereby indicating that the peptide is also oriented parallel to the membrane surface at elevated pH [Fig. 1b). The interactions with POPG thereby contrasts with the transmembrane alignment observed in POPC (225 ± 5 ppm; Fig. 1c and reference (Bechinger 1996)].

The membranes with intermediate surface charge density were prepared, matching more closely the situation found in biological systems. Figure 2 exhibits the ^{15}N solid-state NMR spectra of LAH4 at different peptide-to-lipid ratios when investigated in oriented POPC/POPG (3:1 mol/mol ratio) at low pH. For peptide concentrations of 1, 2 and 4 mol % the chemical shift values of 84 ± 4 ppm (Fig. 2a), 80 ± 8 ppm (Fig. 2b), and 86 ± 10 ppm (Fig. 2b), respectively, are indicative that the peptide is aligned parallel to the membrane surface.

The samples investigated in Figs. 1 and 2 were made from supported membranes between planar glass surfaces and hydrated at ~93 % relative humidity. Although this assures an almost complete hydration of the lipid bilayers, it has been observed that the topological equilibria of some peptides can be shifted by sample hydration. These include the hydrophobic C-terminus of Bcl-x$_L$ (Aisenbrey et al. 2007), alamethicin (He et al. 1996), as well as the amphipathic cationinc peptide PGLa (Salnikov and Bechinger 2011). Therefore, we also investigated the membrane alignment of LAH4 in non-oriented fully hydrated phospholipid membranes where, under conditions of motional averaging around the bilayer normal, the resulting powder pattern line shape provides an indicator of the peptide orientation (Bechinger and Sizun 2003; Prongidi-Fix et al. 2007a). The resulting spectral line shapes shown in Fig. 3 also indicate that in the presence of bulk water, LAH4 is oriented along the membrane surface of POPC or POPG bilayers. The $\sigma_{\parallel}$ and $\sigma_{\perp}$ values are 80 ± 5 ppm and 145 ± 5 ppm for POPC, and 74 ± 5 ppm and 144 ± 5 ppm for POPG, respectively, where $\sigma_{\parallel}$ corresponds to the chemical shifts obtained from oriented samples under comparable conditions (Bechinger and Sizun 2003; Hirschinger et al. 2011).

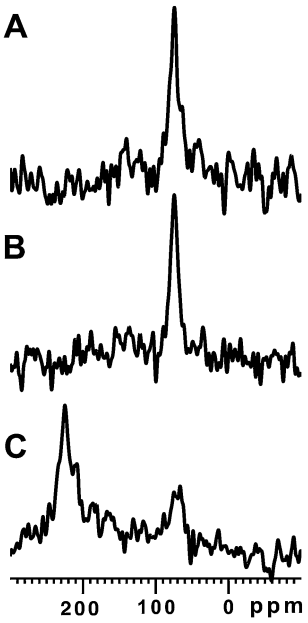

Fig. 1 Proton-decoupled ^{15}N solid-state NMR spectra of 2 mol % [^{15}N-Ala$_{16}$]-LAH4 reconstituted into phospholipid bilayers oriented with the membrane normal parallel to the magnetic field direction in: **a** POPG at pH 5.5, **b** at 7.4 and **c** POPC at pH 7.4. The temperature was 293 K (**c**) or 310 K (**a, b**). The pH was adjusted by the addition of NaOH and/or HCl to the LAH4 aqueous solution

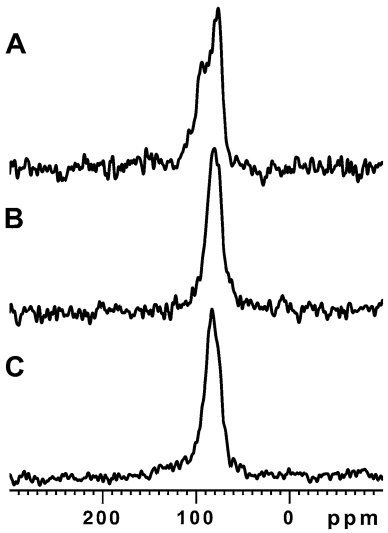

Fig. 2 Proton-decoupled ^{15}N solid-state NMR spectra of [^{15}N-Ala$_{6,16,22}$]-LAH4 (**a**) or [^{15}-N Ala$_{16}$]-LAH4 (**b** and **c**) in POPC/POPG (3:1 mol/mole) bilayers oriented with the membrane normal parallel to the magnetic field direction. The peptide-to-lipid molar ratios are: **a** 1 %, **b** 2 % and **c** 4 %. No buffer was added to these samples and the pH was ~5.5

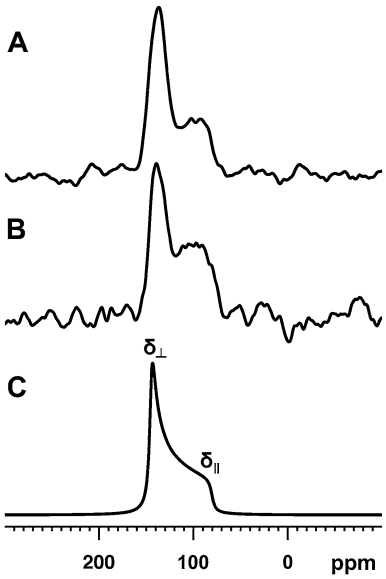

Fig. 3 Proton-decoupled ^{15}N solid-state NMR spectra of [^{15}N Ala$_{16}$]-LAH4 in non-oriented phospholipid bilayers. **a** in POPC, **b** in POPG. The peptide-to-lipid molar ratio is 1:50 and the temperature 310 K. No buffer was added to these samples, therefore, the pH was slightly acidic. **c** Simulated ^{15}N solid-state NMR powder pattern of a helical peptide labelled at a single amide site that is oriented perpendicular to the membrane normal under conditions of fast rotational averaging around this axis. The simulation parameters are: $\delta_{iso} = 120.4$ ppm, $\delta_{11} = \delta_{22} = 143.6$ ppm, $\delta_{33} = 74.17$ ppm; Lorentzian line broadening of 244 Hz

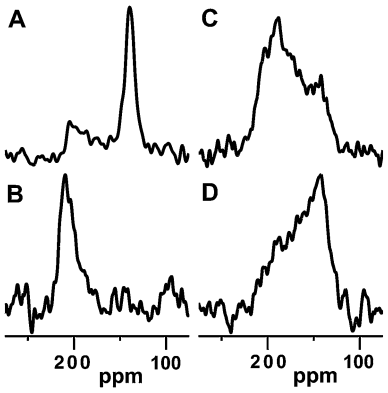

Fig. 4 Proton-decoupled ^{15}N solid-state NMR spectra of [^{15}N-Ala^{16}]-LAH4 in dimyristoyl-phospholipid bilayers oriented with the membrane normal parallel to the magnetic field direction. The lipid compositions are: **a** and **b** DMPC, **c** DMPC/DMPG 3:1 mol/mole, **b** DMPG. Sample B was prepared in the presence of citrate buffer at pH 5.5 while no buffer was added to **a, c** and **d**. The peptide-to-lipid ratio is 1:50, the temperature was set to 310 K (**a, b, d**) and 313 K (**c**)

Biophysical investigations on antimicrobial peptides are often performed on model membranes using either 1-palmitoyl-2-oleoyl-phospholipids (e.g., Figs. 1, 2, 3), thought to represent the average fatty acyl composition and thickness of biological membranes, or dimyristoyl-phospholipids, which are also often used for biophysical measurements because of their gel-to-liquid transitions in easily accessible temperature ranges and their commercial availability in various deuterated forms. Therefore, solid-state NMR spectra were also recorded from LAH4 reconstituted into oriented DMPC membranes. At acidic pH a major contribution with a chemical shift of 80 ± 10 and a minor peak around 210 ppm, are observed thereby indicating a predominantly in-plane alignment of the peptide albeit with a small contribution at transmembrane orientations (Fig. 4a). The latter increases when mixed membranes or pure DMPG membranes are investigated (Fig. 4c, d) suggesting that a sensitive equilibrium exists between these two configurations. This equilibrium can be further shifted to transmembrane in the presence of citrate buffer (Fig. 4b).

Oriented SRCD (oSRCD) spectroscopy

Oriented SRCD spectroscopy enables the topological analysis of membrane polypeptides as well as provides information on their secondary structure, whilst requiring a much smaller sample size than solid-state NMR spectroscopy. Using the high flux of a synchrotron light source enables measurements to be made to lower wavelengths than conventional CD spectroscopy, and, most importantly, measurements can be made in the presence of absorbing compounds such as lipids and buffers, that would not be possible with conventional CD spectroscopy. In this case, with high amounts of both lipids and buffers present, it enabled measurements of the peptide transitions (albeit truncated somewhat at the low wavelength end of the spectrum) that could not be measured at all using a conventional CD instrument. The orientation of peptides with respect to the light source/membrane bilayer can be determined based on the spectral characteristics of the peptide (Burck et al. 2008; Wu et al. 1990). Figure 5a shows oSRCD spectra of three peptides, KL14, KALP, and LAH4, associated with phosphatidylcholine membranes. Anhydrous films produced the best oriented samples, but the hydrated samples enabled the changes associated with pH to be examined. LAH4 exhibits its spectra characteristic of in-plane oriented helices when reconstituted in a POPC bilayer at acidic pH. In comparison, KALP and KL14, when reconstituted in POPC bilayers, assume stable transmembrane and in-plane orientations, respectively (Harzer and Bechinger 2000; Hirschinger et al. 2011; Killian 2003). In contrast, when citrate was added to the LAH4 sample, the spectra changed and became characteristic of peptides with transmembrane helices at pH 5 or pH 7 in both POPC or DMPC membranes (Fig. 5b, c). The temperature dependences of the CD spectra indicate that adoption of the transmembrane alignment requires a liquid crystalline disordered state (above the phase transition) of the phosphatidylcholine bilayers (Fig 5c).

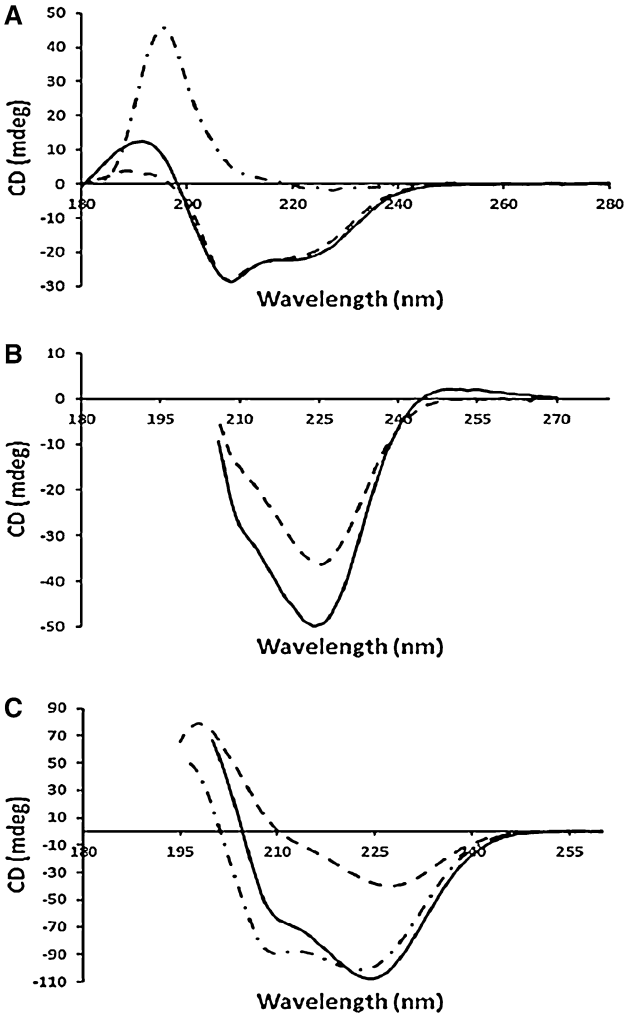

Fig. 5 Oriented circular dichroism spectra of membrane films containing 2 mol % peptide. **a** SRCD spectra of POPC membranes prepared in the presence of KALP (*dashed-dot line*), KL14 (*dashed line*) and LAH4 (*solid line*). No buffer was added and the pH was ~5. **b** CD spectra of POPC membranes with LAH4. The pH was adjusted by addition of citrate/citric acid buffer at pH 5 (*solid line*) or sodium phosphate mono acid/citric acid buffer at pH 7 (*dashed line*) before the samples were dried and equilibrated at 95 % r.h. **c** CD spectra of DMPC membranes with LAH4 where the pH was adjusted to 5 by addition of citrate buffer. The samples were either dried and measured at 313 K (*solid line*) or 283 K (*dash-dotted line*) or hydrated at 95 % r.h. and measured at 313 K (*dashed line*)

Discussion

pH-dependent membrane topology

Previous investigations on the membrane interactions of LAH4 have been performed after the peptide has been reconstituted into POPC membranes. Whereas some of those studies characterized the effects of LAH4 on the phospholipid macroscopic phase properties, as well as the lipid molecular order parameters (Mason et al. 2006c, 2007b), others focused on the peptide structure and membrane alignment using NMR techniques (Bechinger 1996; Bechinger et al. 1999; Georgescu et al. 2010). In POPC lipid bilayers LAH4 acts like a molecular switch where the peptide changes from an in-plane orientation at pH <5.5 to a transmembrane alignment at neutral pH (Figs. 1c and 6). These topological changes are paralleled by structural modifications whereby the helical domain shifts from a carboxy-terminal (residues 9–24) at pH 4.1 to a more amino-terminal localisation (residues 4–21) at pH 7.8 (Georgescu et al. 2010). At pH 6.1 two shorter helices are interrupted by a hinge region encompassing residues 10–13. This flexible domain probably facilitates the membrane insertion during the in-plane to transmembrane transition.

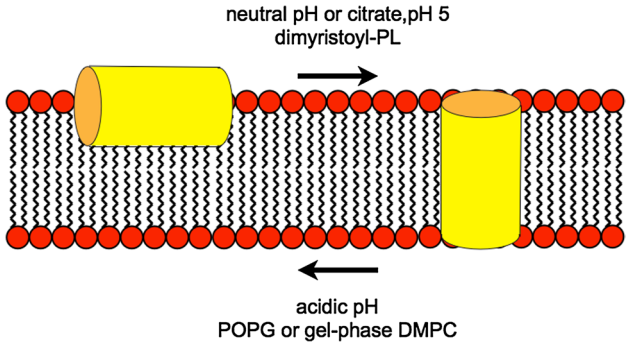

Fig. 6 Schematic drawing of the pH-dependent equilibrium between the LAH4 in-planar and transmembrane alignments

Comparing effects of lipid head groups and buffer composition

When cationic amphipathic peptides, including LAH4, were studied, important functional differences were observed in zwitterionic and negatively-charged phospholipid bilayers (Cheng et al. 2011; Mason et al. 2006b, 2007a; Matsuzaki et al. 1991). For example, the channel-forming activities of LAH4 were less pronounced in the presence of POPG than in POPC (Vogt and Bechinger 1999), but this difference was not correlated to structural changes.

Here, topological information has been obtained for LAH4 in PG membranes. The ^{15}N solid-state NMR investigations indicated that at low pH, and in the absence of citrate, LAH4 lies in-plane in the presence of either POPC or POPG membranes (Figs. 1, 2, 3). Notably, the measurements in fully hydrated liposomal suspensions (Fig. 3) confirm the membrane topology of LAH4 that had previously only been studied using supported lipid bilayers (Bechinger 1996). When the pH is increased >6 the LAH4 topological equilibria in POPC bilayers shifts towards a transmembrane alignment (Figs. 1c, 5b) (Bechinger 1996; Bechinger

et al. 1999) whereas the peptide remains associated with the surface of POPG at neutral pH (Figs. 1b, 6).

The oSRCD and oCD investigations confirm the helical secondary structure of the peptides and the pH-dependence of the in-plane and transmembrane topologies of LAH4 in phosphatidylcholine membranes (Fig. 5a, b). Interestingly, the oCD measurements also show that in the presence of citrate buffer, the transmembrane alignment is stabilized even at acidic pH (Figs. 5b and 6). This may be due to binding of the citrate to the histidines and/or lysines thereby neutralizing the cationic charges in the central region of the helix. Furthermore, the spectra recorded as a function of temperature indicate that the transmembrane alignment is only observed in a liquid disordered bilayer phase (Fig. 5c).

Topological effects of the fatty acyl chain composition

The in-plane topology of the peptide in pure DMPC (Fig. 4a) confirms the alignment under acidic conditions that has been observed previously in POPC membranes by ATR-FTIR and solid-state NMR spectroscopies [Fig. 3a and references (Bechinger 1996; Bechinger et al. 1999)]. In contrast, a partially transmembrane alignment is observed by solid-state NMR spectroscopy in the presence of DMPG (Fig. 4c, d) revealing a pronounced difference between POPG and DMPG membranes. Whereas POPG stabilizes in-plane orientations even at high pH (Fig. 1b), the presence of DMPG facilitates transmembrane insertion even at low pH (Figs. 4c, d). Related differences in membrane topology with fatty acyl chain composition have recently been monitored with PGLa, a linear cationic antimicrobial peptide of 21 residues [reviewed in (Kemayo Koumkoua et al. 2014)].

Interactions influencing membrane topology and function

In order to describe the topology of membrane polypeptides, we consider a number of interactions that contribute differentially to the Gibbs free energy of peptide sequences in bulk solution, in the interface or in the membrane interior (Huang 2000). These contributions include the hydrophobic energy of amino acid side chains and the peptide backbone (White and Wimley 1999) as well as changes in the membrane packing and curvature strain upon peptide insertion (Israelachvili et al. 1980; Kollmitzer et al. 2013; Strandberg et al. 2012). The equilibrium between the in-plane and transmembrane orientations in POPC (Fig. 6) appears to be governed by the energy that is required to neutralize/charge an amino acid at a given pH [discussed in the context of LAH4 realignment in (Bechinger 1996)], a contribution that can be modulated by multivalent counter ions such as citrate (Figs. 4b and 5b). Furthermore, hydrophobic interactions of the amino acid residues when exposed to the membrane interface or bilayer interior, and other contributions such as those that arise when the lipid packing changes during the peptide re-alignment, should be taken into consideration (Aisenbrey et al. 2006a, b; Bechinger 1996).

A new level of complexity is introduced when the peptide is studied in POPG membranes where in-plane alignments are stabilized (Fig. 1b). This can be due to the smaller head group size of this phospholipid (cf. below) and due to electrostatic interactions between the cationic peptide and the anionic PG head group. Furthermore, the negative membrane surface charge density introduced by the PG head groups attracts the positively charged H^+ and repels the OH^- ions, thus, the local pH may be lower in its proximity, which can shift the apparent pK_a of the LAH4 histidines.

Additional energetic contributions can arise from the insertion of amphipathic helices into the membrane interface and the resulting curvature strain on the lipid packing (Bechinger 2009; Salnikov et al. 2009). The latter is released during the transition into a transmembrane alignment. In this context it is interesting to note that the topologies of LAH4 in POPC or POPC/POPG membranes contrast with those in dimyristoyl-phospholipids where, regardless of the lipid head group tested, a more inclined or even a transmembrane alignment is favoured (at pH 5; Fig. 4). More stable in-planar topologies of amphipathic peptides have been correlated with the much smaller size of the head group of phosphatidylethanolamine lipids (Matsuzaki et al. 1998; Salnikov et al. 2010). Along the same line, a more tilted membrane insertion of an amphipathic helical peptide into di-saturated phosphatidylcholines has been explained by differences in the molecular shapes of DMPC and POPC lipids (Strandberg et al. 2013). In this model the size of the lipid head group and the volume occupied by the fatty acyl chains are compared to each other, which results in an inverted cone shape for POPE, a cylinder for POPC, and a slightly cone shaped DMPC lipid. Correspondingly, membranes formed by cone shaped lipids exhibit an inherent negative (POPE) or positive curvature strain (DMPC). Depending on the membrane lipid composition, the additional space taken up by the peptide in the head group region can compensate or enhance this curvature strain (Bechinger 2009). However, recent SAXS measurements indicate that the intrinsic curvature strain of phosphatidylcholines exhibits only a comparatively small dependence on the fatty acyl chain composition (Kollmitzer et al. 2013) suggesting that this is not the only contribution favouring a more inserted topology of some peptides in DMPC. Possibly, additional energetic contributions arise from the kink in the unsaturated fatty acid tail of POPC and POPG, that allows the palmitoyl-oleoyl lipids to compensate the peptide-imposed curvature strain with less entropic penalty.

Conclusions

In POPG membranes the LAH4 peptides are about six-times more active in calcein release experiments at low pH than when compared to pH 7.4 (Vogt and Bechinger 1999). Here, we show that under both conditions, the peptide is aligned parallel to the membrane surface suggesting that not only the membrane alignment but other factors, such as the depth of penetration into the bilayer interface, play a role in membrane activity and the associated antimicrobial action. The subtle interplay between the fatty acyl chain saturation, head group size and charges may tune the interactions between the lipids and the peptides. In summary, LAH4 exhibits a highly dynamic character with its topological location not only governed by pH but also the presence of counter-ions, the lipid head group, and fatty acyl chain composition.

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