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Review

Detergent-like actions of linear amphipathic cationic antimicrobial peptides

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Abstract

Antimicrobial peptides have raised much interest as pathogens become resistant against conventional antibiotics. We review biophysical studies that have been performed to better understand the interactions of linear amphipathic cationic peptides such as magainins, cecropins, dermaseptin, δ-lysin or melittin. The amphipathic character of these peptides and their interactions with membranes resemble the properties of detergent molecules and analogies between membrane-active peptide and detergents are presented. Several models have been suggested to explain the pore-forming, membrane-lytic and antibiotic activities of these peptides. Here we suggest that these might be 'special cases' within complicated phase diagrams describing the morphological plasticity of peptide/lipid supramolecular assemblies.

Keywords: Polypeptide lipid interaction; Peptide pore formation; Phospholipid membrane; Bilayer; Regulation; Selectivity

Contents

The increasing resistance of pathogens against many commonly used antibiotics requires urgent actions and the continuous research and development of new bactericidal and fungicidal compounds [1,2]. An interesting approach consists in searching for naturally occurring antibiotic molecules and both the plant and animal kingdom are rich sources. Antibiotic peptides have been isolated from amphibians or insects as early as 1962 [3], but this field of research has attracted considerably more attention during the last two decades. Both plants and animals produce, store and secrete antibiotic peptides in exposed tissues, or synthesize such compounds upon induction. The availability of these molecules establishes a defense system that can be set into action immediately when infections occur [4–8]. Meanwhile the number of antimicrobial peptides identified in nature is continuously increasing and compiled in the Antimicrobial Sequences Database (http://www.bbcm.univ.trieste.it/~tossi/antimic.html). By now nearly 1000

Abbreviations: DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DOPA, 1,2-dioleoyl- *sn*-glycero-3-phosphatidic acid; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; NMR, nuclear magnetic resonance; PC, 1-2-diacyl-*sn*-glycero-3-phosphocholine; PE, 1,2-diacyl-*sn*-glycerol-3-phosphoethanolamine; PG, 1,2-diacyl-*sn*-glycerol-3-phosphoglycerol; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol

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peptides have been identified and many more have been created by design using also synthetic combinatorial library approaches [9]. Understanding the structure–function relationship of these peptides allows for the design of cheaper and more efficient analogues [10,11]. In general the peptides exhibit broadband antimicrobial activities, when at the same time the detailed spectrum of antibiotic and antifungal activities varies with their amino acid sequence. Antimicrobial peptides can also exhibit virucidal and tumoricidal activities [12,13]. The peptide composition and structure thereby reflect the adaptation of the species to their particular microenvironments.

The focus of this review will be on linear amphipathic peptides which are found in many species including plants, amphibians, insects or humans [14–16]. Some of the best studied linear peptide antibiotics are the members of the magainin family first found in amphibians [17,18], as well as cecropins, which were isolated from the pupae of the cecropia moths [19,20]. Whereas magainins are stored in granula in the skin of frogs, cecropins are induced upon infection.

The amphipathic distribution of polar and hydrophobic residues can result in pronounced interactions of the peptides with phospholipid membranes. Therefore, a common characteristic observed for membrane-active peptides is their capability to disturb bilayer integrity, either by creation of defects, disruption or pore formation (Fig. 1). The resulting openings in the lipid bilayer lead to the collapse of the transmembrane electrochemical gradients and, therefore, provide an explanation of the cell killing activities of these peptides (reviewed e.g. [21,22]). The formation of pores affects cellular respiration [23], deprives sensitive organisms of their source of energy by disrupting the electrochemical gradient across free-energy transducing membranes [24,25] and results in increased water and ion flow across the membrane concomitant with cell swelling and osmolysis [26,27].

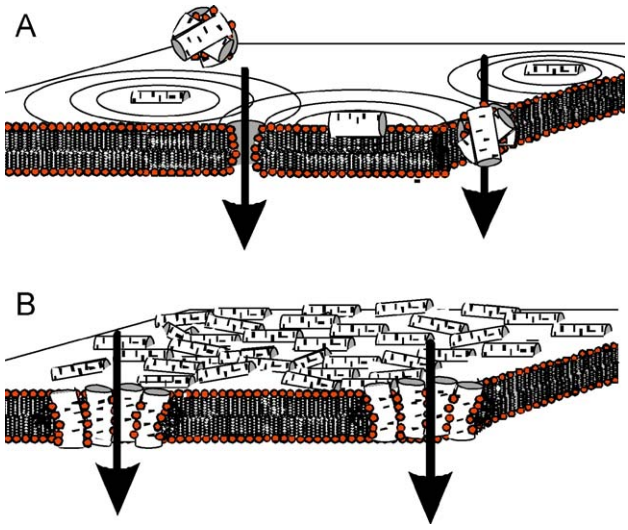

Fig. 1. Models describing the interactions of linear cationic amphipathic peptides with membranes. (A) At low concentrations three effects of detergent-like molecules are shown. A peptide micelle 'hits' the membrane, thereby adsorbing a few lipid molecules concomitant with pore formation (top). The micelle can also 'insert' in the membrane resulting in a 'micellar aggregate channel' (right side). Furthermore, as in the presence of detergents in-plane oriented amphipathic peptides destabilize the membrane within a diameter of several nanometers (circles). Lateral diffusion of the peptides along the membrane surface results in transient changes in local peptide density and the temporary collapse of the membrane ohmic resistance when peptides approach each other. (B) At higher concentrations the membrane disintegrates as described by the carpet-, torroidal pore- or wormhole models.

Interestingly, all-D-magainins, all-D-cecropins, cecropins with inverted sequences (retro) or inversed D-cecropins (retro-enantio), all possess the high antibiotic and the pore-forming activities of the parent L-enantiomer [28–30]. Moreover, both the L- and D-enantiomer of protegrin-1 showed the same effect on membrane model systems composed of neutral or charged phospholipids [31]. Furthermore β-amino acid oligomers [32] or helical pseudo-peptides show antibiotic activities in the same concentration range as observed for most active antimicrobial peptides [33]. These observations indicate that the cell killing activities of such amphipathic peptides are not mediated through specific, chiral receptor interactions, but are mostly due to direct peptide–lipid interactions. This is in agreement with initial observations that permeabilization of microbial membranes is an early and necessary step in killing microbes as demonstrated for magainin [24] and human defensin [34]. Further support comes from electron microscopy [35] and atomic force microscopy [36] studies on *E. coli* cells exposed to magainin or to PGLa, another member of the magainin family, which show that the lethal event is rupture of the inner membrane. Indeed systematic investigations demonstrate that the interplay between overall charge, hydrophobicity and hydrophobic moment is important for selectivity and antibiotic activity of amphipathic helical model peptides [37–41]. However, it should be noted that more recent investigations give evidence that the antibiotic activity may also develop due to interactions of these peptides with intracellular targets (for a review see [12,42]). Nevertheless, also in these cases the membrane has to be crossed to allow penetration into the cell interior. Importantly many linear peptides, such as those of the magainin family, adopt amphipathic structures only when in contact with membranes, membrane-mimicking environments [43,44] or when aggregated [45]. As a matter of fact this transition is important for activity as it provides about 50% of the energy of membrane association [46,47]. It is therefore important to study their structures in their appropriate environments.

1. Models for membrane disruption by antimicrobial peptides

Regarding membrane permeation and disruption two distinct mechanisms have particularly been presented and discussed. One model proposes that antimicrobial peptides act by perturbing the barrier function of membranes through transmembrane pore formation [48], while the second model suggests membrane disruption via the so-called "carpet" mechanism [49]. As these modes of action of antimicrobial peptides will be discussed in more detail elsewhere in this special issue we shall only briefly describe these models (Fig. 1).

The formation of discrete multi-level openings and reproducible channel characteristics observed with predominantly

hydrophobic peptides such as the fungal peptide alamethicin have been taken as an indication for the formation of transmembrane helical bundles [50]. Similar models have been initially suggested also for charged amphipathic peptide antibiotics such as magainins, cecropins or pardaxin, however, it has soon been realized that these peptides exhibit differences in functional and structural properties. Moreover, it was shown for synthetic pardaxin P1a and Pa4 that membrane perturbation also depends on the membrane composition [51,52] as has been emphasized earlier for the action of antimicrobial peptides [53,54]. Linear cationic peptide antibiotics exhibit erratic currents in electrophysiological experiments, a strong tendency of the membrane to rupture and an ion specificity that is strongly dependent on the lipid composition [55–57]. The formation of other forms of supramolecular structures in the membrane has, therefore, been suggested for these peptides.

1.1. The wormhole- or torroidal pore model

This model is an extension of the transmembrane helical bundle in which the pores are lined with peptides and lipids (Fig. 1B, front). Such a pore structure was first proposed by Matsuzaki et al. [58] based on the observation that magainin 2 induces rapid lipid flip-flop coupled with pore formation. The presence of negatively charged phospholipids in the pore-lining reduces the repulsive interactions due to the high positive charge-density of the peptides [21] and could explain modulations in ion selectivity due to changes in the membrane lipid composition [56]. Indeed at high magainin concentrations (>3.3 mol% corresponding to >10 wt.%) neutron in-plane scattering on mechanically aligned membrane systems indicates the presence of water filled cavities, which resemble 'wormholes' [47,59]. The model thus postulates that peptides and lipid form together well-defined pores [60]. The arguments in favour or contradicting such a mechanism have been discussed in considerable detail previously [21,61].

1.2. Carpet model

Based on experimental results obtained with the antibiotic peptide dermaseptin the carpet model has been proposed [19,22,62]. This 34-residue antibiotic peptide is rich in lysines and adopts an amphipathic α-helix (residues 1–27). It thereby resembles cecropins and magainins for which an in-plane alignment had been demonstrated [63,64]. The peptide partitions into acidic and zwitterionic membranes [62]. In the presence of negatively charged lipids and at high peptide concentrations dermaseptin is located at the membrane surface and it has been suggested to self-associate in a 'carpet-like' manner (Fig. 1B, back). The model further suggests that the membrane breaks into pieces when a threshold concentration of peptide is reached [65]. The presence of negatively charged lipids helps in the formation in a dense peptide carpet, as they help to reduce the repulsive electrostatic forces between positively charged peptides.

Whereas the in-plane orientation of these peptides reflects the detergent-like properties of these amphipathic peptides, the carpet model describes the lysis of the membranes at high local peptide concentrations and reflects the morphological changes of the membrane after a threshold concentration at the membrane surface is reached (Fig. 2). Although high peptide densities have been observed at the surface of bacterial cells, the concentrations at the membrane itself remain a matter of speculation [66]. Furthermore, membrane-activities have been demonstrated at reduced peptide-to-lipid ratios. Indeed comparatively low peptide-to-lipid ratios are needed to dissipate the ionic gradient across cell membranes or to develop antibiotic activity [25,37,67]. Notably, it has been speculated that small openings are sufficient for ion release concomitantly with inhibition of respiration [25] when at the same time bigger pores and higher peptide-to-lipid ratios are required for osmolysis of red blood cells. In a similar manner single-channel recordings of cecropin or magainin require small amounts of peptide within the membrane as otherwise lysis occurs [55,56,68]. We therefore prefer to describe the activities of these peptides by a more general model which is based on their detergent-like properties [69–71].

1.3. The 'detergent-like' model

Represents the most generally applicable explanation for the membrane-activities of amphiphiles. The model is based on the intercalation of these molecules into the bilayer (Fig. 1A). Thereby, the nature of the molecules such as charge and hydrophobic volume strongly contribute to the variety of actions observed and the lipid polymorphism induced [72]. The interaction of detergents with lipid membranes in particular above the critical micelle concentration (cmc), where the molecules exist in their aggregated, micellar state is rather complex. However, it has to be noted that antimicrobial peptides can also exist as oligomers (Fig. 1A, top) and thus may exhibit different behaviour as compared to the monomeric peptides (see below). Let us consider those general features of detergent–lipid interactions, which are more related to the action of antimicrobial peptides. Whereas detergents at very low detergent-to-lipid ratios can have a neutral effect on model membranes, or can even result in their stabilization [73,74], openings might form temporarily at intermediate concentrations, and membrane disintegration becomes apparent at higher peptide-to-lipid ratios (Figs. 1B and 2). Disintegration of the bilayer takes into account the loss of the membrane barrier, dissipation of the transmembrane electrochemical gradient, loss of cytoplasmic constituents and concomitantly interference with the energy metabolism of living cells, all being observed also with antimicrobial peptides. However, it remains possible that, depending on the peptide and membrane composition, the peptides merely form small and transient openings (Figs. 1A and 2) which suffice for the diffusion of smaller molecules, including the peptides themselves in and out of the cell interior. The mechanism by which antimicrobial and cytolytic peptides finally damage the membrane will be determined by the peptide and lipid structure [42].

The step-wise changes in conductivity that have been observed for magainin 2 [55–57] or for cecropins [75] are

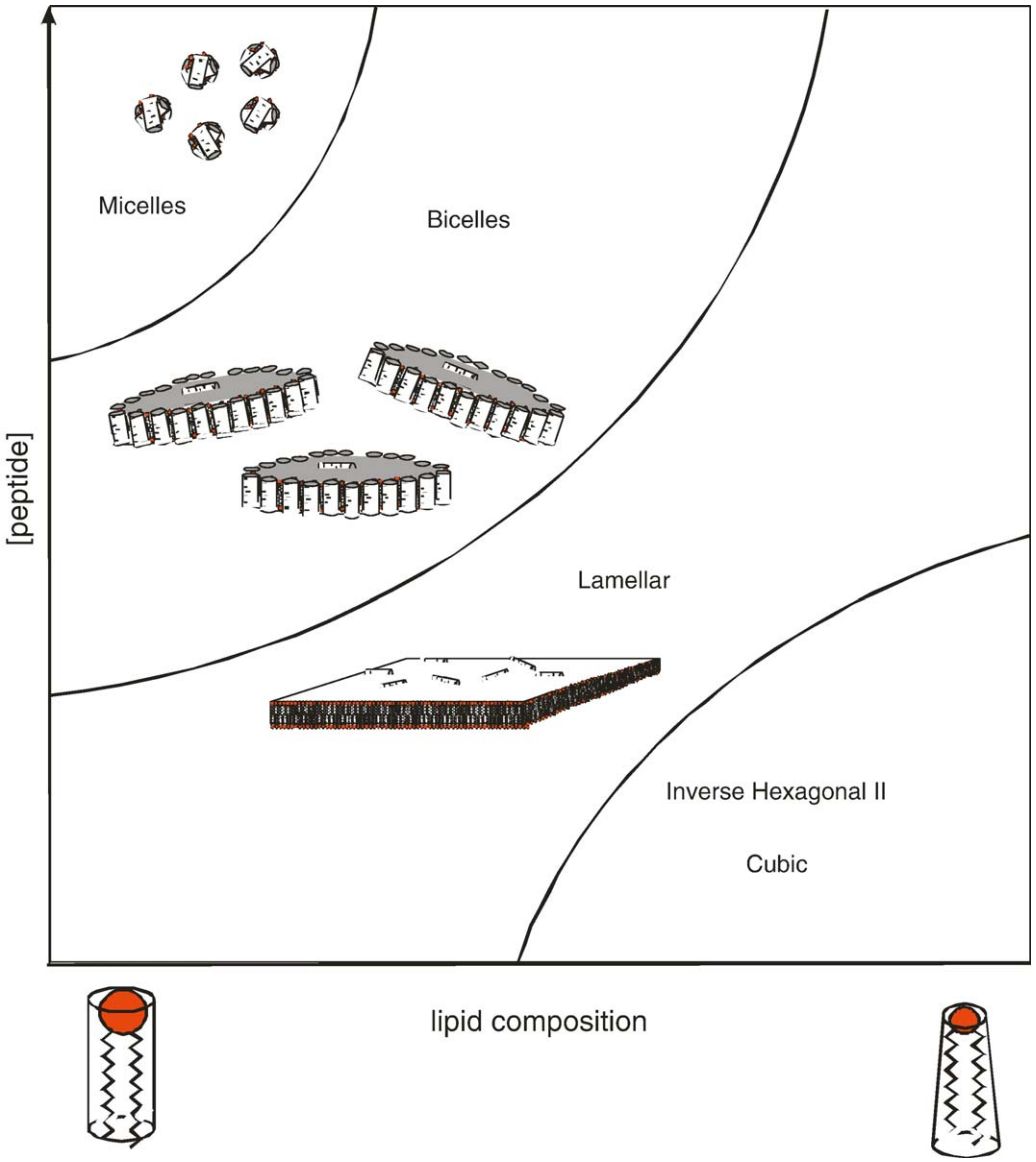

Fig. 2. This figure shows a schematic phase diagram of antibiotic peptide/phospholipid mixtures. A variety of macroscopic phases are obtained as a function of peptide/lipid ratios and lipid composition. Here two lipids with different molecular shapes are mixed and their effect on the macroscopic phase transition in the presence of antibiotic peptides is shown. For a complete description of antibiotic activities other parameters such as temperature, pH, or cholesterol concentration would have to be considered.

puzzling observations, which are on first view difficult to reconcile with an alignment of these peptide helices along the membrane interface. Interestingly, however, step-wise conductivity increases have also been observed in the presence of detergents [76,77], in pure lipid membranes [78–80], or when small unilamellar phospholipid vesicles are added to planar lipid bilayers [81]. On a molecular level one could thus imagine that the temporal and local fluctuations of the peptide/detergent density, related to their lateral and translational diffusion along interfacial localizations, causes stochastic accumulations of peptide/detergent in time and space. When high enough local densities are reached a decrease in ohmic resistance occurs [21]. Alternatively, electroporation of the membrane has been suggested for NK-lysine an amphipathic helical peptide lying on the surface of the membrane. In this case the high local electric fields are sufficient for transient pore formation [82].

An interesting feature of the detergent-like model is the potential aggregation of the peptides into micelles in aqueous solution (Fig. 1A, top), whereby an equilibrium between monomeric peptides and peptide aggregates has to be taken into account that will result in concentration dependent effects. For example, tetrameric aggregates have been observed for melittin under specific conditions [83] and larger aggregates depending on pH and peptide concentration have been reported for δ-lysin [84,85]. Self-assembly of peptides was also observed for natural and synthetic acylated antimicrobial peptides [86–88]. Such peptide micelles might interact with the lipid bilayer, e.g. by carrying away lipid molecules which would also result in transient openings. On the other hand such peptide micelles could insert (or form) in the lipid bilayer (Fig. 1A), thereby forming structures that resemble pores without, however, being well defined in shape or size [89]. The lipopeptaibol trichogin

GA IV isolated from the soil fungus *Trichoderma longibrachiatum* [90] may be an example for such a mechanism. Leakage and light scattering experiments suggest that within the explored concentration range membrane perturbation of this peptide is due to pore formation of the peptide aggregates and not to micellization [88]. Furthermore, the ability to self-associate in aqueous medium may be important for target cell selectivity [86,91]. The role of pre-assembly was tested using monomeric diastereomeric cationic peptides and their covalently linked pentameric bundles [92]. In contrast to the peptide aggregates that expressed similar potent antifungal and high antimicrobial activity as well as haemolytic activity independent on peptide length, the monomeric peptides showed length dependent antimicrobial activity and were devoid of haemolytic activity.

A complete description of the peptide–membrane interactions and the resulting membrane morphologies would need to take into account a wide variety of parameters and conditions. These include the peptide-to-lipid ratio, the detailed membrane composition, temperature, hydration and buffer composition. As with detergents this can be done by establishing phase diagrams (Fig. 2). Notably, the detergent-like properties of amphipathic peptides are not in contradiction but include the above mentioned wormhole and carpet models, in fact these latter models should be considered 'special cases' where the conditions are such that these kind of supramolecular structures are observed within a much more complex phase diagram. It therefore seems tedious to argue about the 'correct model' as all of them might occur depending on the detailed conditions. Notably, the extensive plasticity of peptide/detergent–lipid complexes opens up the possibility that a peptide induces a certain macromolecular structure when interacting with the membranes of one organism, but a different one when interacting with another species. On the other hand the mutagenesis of the peptide sequence causes shifts in the phase diagram and therefore also affects the biological mechanisms.

In the following we have assembled a number of experimental results which illustrate the detergent-like properties of amphipathic peptides. When the permeability changes of membranes are investigated the leakage of fluorescence dyes takes place at magainin concentrations of approximately 3 mol % which is equivalent to 81 g/mole lipid. This latter value is very similar to those observed for the permeability increases in the presence of the detergents Triton-X100 and octyl glucoside [76,93].

Furthermore, the macroscopic phase properties as monitored by proton-decoupled ^{31}P solid-state NMR spectroscopy exhibits interesting parallels when amphipathic peptides, short chain lipids, lysolipids and detergents are compared to each other [70,71]. Pore formation due to the increased membrane tension has also been suggested for other amphipathic helical peptides [94] and cecropin B activity on bacterial cells was compared to that of quartenary detergents [95].

When the molecular shape of amphipathic helices and detergents are compared to each other it becomes evident that, when intercalated into a lipid bilayer, neither of them fills the volume at the level of the fatty acyl chains and in the lipid head group region equally well (Fig. 3). Whereas the peptide acts as a spacer at the level of the lipid headgroup it does not occupy the corresponding volume at the level of the fatty acyl chains creating a void in the hydrophobic region of the membrane bilayer [21,42,70]. This parallels the behaviour described for charged alkyl compounds, which are one of the simplest, among the variety of detergent molecules being also anchored at the lipid–water interface with their polar headgroup (for a review see [72]). Systematic studies using PC model membranes revealed that molecules with short alkyl chains (C_{6-12}) are located in the more ordered plateau region of the acyl chain region of the phospholipids inducing voids below their terminal methyl groups. Since the formation of such free volume is energetically unfavourable [96], the hydrocarbon chains must eliminate these voids by chain bends, increased *trans-gauche* isomerization [97] or chain interdigitation as found for very short chain amphiphiles like alcohols (e.g. [98]) thus affecting the phospholipids' acyl chain packing. Similar effects were observed also for non-ionic surfactants such as *N*-alkyl-*N,N*-dimethylamine-*N*-oxides [99] which were shown to exhibit antimicrobial activity [100,101]. Increasing the size of the polar headgroup would lead to a molecular shape of the compound that can be described by a cone which would further promote the formation of voids.

Amphipathic peptides thus exhibit effects similar to those observed with cone- or wedge-shaped molecules. Intercalation of peptides into the membrane interface therefore creates voids

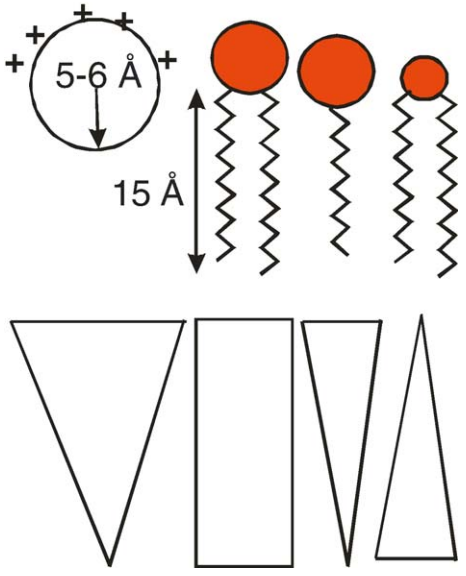

Fig. 3. The molecular shape of lipids and in-plane oriented amphipathic peptides is illustrated. Whereas lipids such as POPC are considered to exhibit a cylindrical overall molecular shape, the decreased size of the PE head group results in an inverted cone structure. On the other hand monoacyl-phospholipids, detergents or in-plane oriented amphipathic peptides predominantly fill the space at the head group region/lipid interface thereby resembling a cone. This model should be considered only as a first order conceptual approach to the real situation. Notably, the 'molecular shape' of the amphipathic molecule reflects the geometrical as well as the interaction space of these molecules. Therefore charge and hydrogen interactions between the lipids and the peptide have to be considered. Furthermore, in-plane oriented peptides can reside as monomers or as side-by-side oligomers in the membrane (e.g. [125]).

in the hydrophobic membrane region and in addition imposes curvature strain on the lipid bilayer [102]. On the other hand the head group of phosphatidylethanolamine occupies a smaller area in the interfacial region when compared to the hydrophobic membrane interior. Therefore, this lipid represents an inverted cone structure. Due to their shape, mixing cone and inverted-cone molecules release the strain and lipid bilayers that exhibit a tendency to form hexagonal phases e.g. at increased temperatures are stabilized by the insertion of amphipathic peptides [37,103]. Stabilization of phosphatidylethanolamine bilayers was also observed for a number of detergents such as Triton X-100, deoxycholate or octylglucoside which exhibit a cone shaped molecular geometry [73].

Considering the large variety of conditions it seems impossible to establish a complete phase diagram for peptide–lipid interactions. However, over the years a considerable amount of data has been collected for melittin. Its overall amphipathic character composed of a cluster of cationic amino acids at the C-terminus and a stretch of hydrophobic amino acids, resembles the features of many detergents being characterized by a polar/charged headgroup and a hydrophobic moiety. This peptide is too lytic to be used as an antibiotic but it can nevertheless illustrate the complexity of such interactions. There is experimental evidence that melittin inserts into the membrane interface [104]. The possibility remains that a small proportion also adopts a transmembrane alignment in the presence of POPC but not POPG [105,106]. Melittin was found to exhibit pronounced effects on the phase behaviour of DPPC already at very low peptide concentrations (lipid-to-peptide molar ratio of 1000/1) [107,108]. A similar concentration dependent behaviour was reported for the detergent cetyltrimethylammonium chloride [74]. Since these effects cannot be accounted for only by local perturbations around the sites of interaction, long range effects beyond the immediate neighbourhood of the incorporated peptide must be involved. Therefore, it was suggested that the peptide-affected domains create line defects in an ordered lipid lattice [104]. Such a defect-like action at low peptide concentrations may be explained by shifting the percolation balance of coexisting gel- and fluid-like states [109]. However, at high melittin concentrations (lipid-to-peptide molar ratio of 15/1), disk-shaped particles were found for the DMPC/melittin mixture [110,111], suggesting a detergent-like solubilization of the membrane under these experimental conditions. Below the gel to fluid phase transition of the pure lipid the presence of intermediate amounts of melittin (<5 mol%) results in the reversible disintegration of the bilayer into disks (cf. Fig. 2) [110,112,113]. It should be noted that some of the mixed peptide/phospholipid phases exhibit meta-stable properties. Therefore in the presence of melittin or magainins the transitions between one phase to another might take many hours even months [70,114, A. Ramamoorthy, personal communication].

The idea of gradual membrane disintegration is also supported by data gained on δ-lysin/PC multilamellar vesicles [115,116]. This peptide from *S. aureus* is, like melittin, 26 residues in size and surface-active. It lyses erythrocytes and many other types of mammalian cells, as well as intracellular organelles and bacterial protoplasts [117]. Similarly to the DPPC/melittin system, the addition of small quantities (lipid-to-peptide molar ratio ≥1000/1) of peptide had only minor but significant effects on the phase behaviour of DPPC or DMPC bilayers. Increasing the peptide concentration to molar lipid-to-peptide ratios lower than 125/1 promoted the formation of two populations of lipid particles, which could be separated by centrifugation. Thereby, small-angle X-ray scattering measurements confirmed that the pellet consists of multilamellar vesicles, while disk-shaped lipid–peptide aggregates were found in the supernatant, the predominant aggregate form at high peptide concentration [116]. Interestingly, the structural parameters of the DMPC/δ-lysin discoidal aggregates (diameter of about 14 nm and a bilayer thickness of 5.2 nm) correspond well with the hydrodynamic radius of 6.9 nm estimated for the disk-shape particles of DMPC/melittin from gel-filtration experiments [110]. Modelling of the X-ray data suggested a peptide ring of about 1 nm thickness surrounding the discoidal lipid bilayer (illustrated in Fig. 2). These data support the idea that lytic peptides like detergents may have concentration-dependent effects on the membrane structure inducing bilayer perturbations of long-range order at low peptide concentrations and exhibiting a detergent-like solubilization of the membrane at high peptide concentrations. Pore formation, which has also been reported for δ-lysin [118], is not necessarily in contradiction to this proposal, but may either reflect a transient state or occur at distinct environmental conditions (Fig. 2). Pore formation might be particularly sensitive to the lipid class composing the membrane. Whereas melittin and δ-lysin disrupt PC bilayers into small structures, the peptides exhibit bilayer-stabilizing effects when mixed with phosphatidylethanolamine membranes, which in the absence of peptide show a tendency for the inverse hexagonal phase (H_{II}) [119].

A different behaviour is observed when melittin is inserted into charged phospholipids such as cardiolipin, DOPA or egg-PG. In these cases a preference for inverted macroscopic phases is observed (H_{II} or cubic) [120,121]. Fluorescence spectroscopy indicates that the macroscopic phase properties depend not only on the molecular shape of the lipid but also on the insertion depth of the peptide [122]. Interestingly, the presence of negative surface charge densities stabilizes PC lipid bilayers in the presence of melittin [106,111,123,124]. Thus the positioning and topology of melittin, and concomitantly its effect on membrane structure, are all strongly dependent on a wide variety of parameters that determine the local environment.

Much less data are available for the peptide–lipid interactions involving antibiotic peptides. However, comparable correlations between the molecular shape of the phospholipids and macroscopic phase transitions have been observed for magainin or its analogue MSI-78 [70,71]. The peptides have been shown to be oriented parallel to the membrane surface using solid-state NMR spectroscopy [64] and MSI-78 to form antiparallel dimers, when solubilized in dodecylphosphocholine (DPC) micelles [125]. The latter observation suggests that magainin activity may be also modulated by oligomerization. Fluorescence energy transfer measurements indicate that the hydrophobic regions of

magainin 2 are located approximately 10 Å from the bilayer center [126]. The peptide or its analogue MSI-78 thus acts like a wedge, which augments the curvature of the membrane. By inserting in-plane peptides into the headgroup region the bilayer packing is disturbed at an estimated radius around the peptide of approximately 50 Å resulting in a reduction in the average bilayer thickness [127–130]. A current atomic force microscopy study on DMPC bilayers and the magainin analogue MSI-78 also revealed that the membrane thickness is not reduced uniformly over the entire bilayer area [130]. Deuterium or ^{13}C solid-state NMR measurements indicate a decrease in order parameter at the lipid bilayer interior for both magainin and MSI-78 [131,132]. More recent investigations indicate that in mixed PC/PS lipid membranes cationic amphipathic peptides preferentially interact with the acidic phosphatidylserine [133,134]. A preferential interaction with the negatively charged lipid component in mixed model membranes was also observed earlier for human neutrophil peptide, HNP-2 [135], PGLa [53] or nisin [136] resulting in charge segregation. Such a behaviour can be expected because of the cationic nature of the peptides, and is typical for a large number of antimicrobial peptides, such as e.g. the α-helical peptides belonging to the magainin-family [35,137] and buforin II [138], the β-sheet peptides tachyplesin [139] and protegrin-1 [54,140], the cyclic peptides gramicidin S [141] and rhesus theta defensin, RTD-1 [142] as well as nisin Z [143]. There is also evidence that other biologically active amphipathic molecules, such as cardiotoxin [144] and synthetic peptides [145] interact with model membranes inducing lateral separation of phospholipids into co-existing domains.

Additional support for the detergent-like model arises from the observation that antibiotic or model peptides too short to span the membrane exhibit channel-like activities as well [146–153]. In particular the model is in perfect agreement with the experimentally observed in-plane orientation of amphipathic peptides [45,64,126].

The membrane lipid composition can thus have pronounced effects on the membrane-disrupting activities of amphipathic peptides. This is reflected by shifts in the borders within the peptide–membrane phase diagram or by the occurrence of different macroscopic phases when one diagram is compared to another. The lipid composition thereby modulates the sensitivity of the membrane to a given peptide [42,53,54,154]. For example, the alignment and the dynamics of the C-terminal helical domain of pardaxin are a function of the phosphatidylcholine fatty acyl chain composition [51,52]. Furthermore, cholesterol which is only present in eukaryotic cells changes the interactions of cationic peptides with the membrane [68,155]. Cholesterol affects the fluidity and the dipole potential of phospholipid membranes. Furthermore, its capacity to form hydrogen bonds with the peptide has also been suggested to reduce antibiotic activity [156]. However, recent experiments on δ-lysin showed that in ternary lipid systems mimicking mammalian cell membranes the peptide binds preferentially to the liquid-disordered domain suggesting that there is no specific interaction between δ-lysin and cholesterol and sphingomyelin, respectively, emphasizing the importance of membrane properties in lipid–peptide interactions [157]. As a result of peptide accumulation peptide aggregates may form that become orientationally ordered, which in turn may have an impact on several processes such as peptide translocation across membranes [158]. Moreover, peptide enriched lipid domains will form especially in the presence of anionic lipids that differ significantly in their local properties from the membrane bulk phase [42]. This may result in packing defects giving rise to increased membrane permeability. In addition, exclusion of certain lipids, e.g. segregation of anionic lipids, from areas of the cell membrane due to their preferential interaction with the cationic peptides may affect membrane structure and integrity [133,134]. Thus, it is evident from the discussion above that the molecular mechanism of membrane permeation and disruption obviously depends on a number of parameters such as the nature of the peptides and membrane lipids, peptide concentration and environmental conditions.

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References

[1] K. Lohner (Ed.), Development of Novel Antimicrobial Agents: Emerging Strategies, Horizon Scientific Press, Wymondham, Norfolk, UK, 2001.
[2] R. Novak, B. Henriques, E. Charpentier, S. Normark, E. Tuomanen, Emergence of vancomycin tolerance in *Streptococcus pneumoniae*, Nature 399 (1999) 590–593.
[3] G. Kiss, H. Michl, Über das Giftsekret der Gelbbauchunke *Bombina variegata* L, Toxicon (Oxford) 1 (1962) 33–39.
[4] E.S. Kristyanne, K.S. Kim, J.M. Steward, Magainin 2 effects on the ultrastructure of five plant pathogens, Mycologia 89 (1997) 353–360.
[5] L. Cavallarin, D. Andreu, B. San Segundo, Cecropin A-derived peptides are potent inhibitors of fungal plant pathogens, Mol. Plant-Microb. Interact. 11 (1998) 218–227.
[6] M. Zasloff, Antimicrobial peptides in health and disease, N. Engl. J. Med. 347 (2002) 1199–1200.
[7] A.R. Alan, E.D. Earle, Sensitivity of bacterial and fungal plant pathogens to the lytic peptides, MSI-99, magainin II, and cecropin B, Mol. Plant-Microb. Interact. 15 (2002) 701–708.
[8] H.G. Boman, Antibacterial peptides: basic facts and emerging concepts, J. Intern. Med. 254 (2003) 197–215.
[9] S.E. Blondelle, K. Lohner, Combinatorial libraries: a tool to design antimicrobial and antifungal peptide analogues having lytic specificities for structure–activity relationship studies, Biopolymers 55 (2000) 74–87.
[10] B. Bechinger, Structure and functions of channel-forming polypeptides: magainins, cecropins, melittin and alamethicin, J. Membr. Biol. 156 (1997) 197–211.
[11] P.M. Hwang, H.J. Vogel, Structure–function relationships of antimicrobial peptides, Biochem. Cell. Biol. 76 (1998) 235–246.
[12] J.P. Powers, R.E. Hancock, The relationship between peptide structure and antibacterial activity, Peptides 24 (2003) 1681–1691.
[13] R.I. Lehrer, Primate defensins, Nat. Rev., Microbiol. 2 (2004) 727–738.
[14] C.L. Bevins, M. Zasloff, Peptides from frog skin, Annu. Rev. Biochem. 59 (1990) 395–414.
[15] H.G. Boman, Antibacterial peptides: key components needed in immunity, Cell 65 (1991) 205–207.
[16] F. Garcia-Olmedo, A. Molina, J.M. Alamillo, P. Rodriguez-Palenzuela, Plant defense peptides, Biopolymers 47 (1999) 479–491.

[17] A. Csordas, H. Michl, Isolierung und Strukturaufklärung eines hämolytisch wirkenden Polypeptides aus dem Abwehrsekret europäischer Unken, Monatsh. Chem. 101 (1970) 182–189.
[18] W. Hoffmann, K. Richter, G. Kreil, A novel peptide designated PYLa and its precursor as predicted from cloned mRNA of *Xenopus laevis* skin, EMBO J. 2 (1983) 711–714.
[19] H. Steiner, D. Hultmark, A. Engstrom, H. Bennich, H.G. Boman, Sequence and specificity of two antibacterial proteins involved in insect immunity, Nature 292 (1981) 246–248.
[20] D. Hultmark, A. Engstrom, H. Bennich, R. Kapur, H.G. Boman, Insect immunity: isolation and structure of cecropin D and four minor antibacterial components from *Cecropia pupae*, Eur. J. Biochem. 127 (1982) 207–217.
[21] 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.
[22] Y. Shai, Mechanism of the binding, insertion and destabilization of phospholipid bilayer membranes by alpha-helical antimicrobial and cell non-selective membrane-lytic peptides, Biochim. Biophys. Acta 1462 (1999) 55–70.
[23] M. Hugosson, D. Andreu, H.G. Boman, E. Glaser, Antibacterial peptides and mitochondrial presequences affect mitochondrial coupling, respiration and protein import, Eur. J. Biochem. 223 (1994) 1027–1033.
[24] H.V. Westerhoff, D. Juretic, R.W. Hendler, M. Zasloff, Magainins and the disruption of membrane-linked free-energy transduction, Proc. Natl. Acad. Sci. U. S. A. 86 (1989) 6597–6601.
[25] L. Silvestro, K. Gupta, J.N. Weiser, P.H. Axelsen, The concentration-dependent membrane activity of cecropin A, Biochemistry 36 (1997) 11452–11460.
[26] W.F. DeGrado, G.F. Musso, M. Lieber, E.T. Kaiser, F.J. Kezdy, Kinetics and mechanism of hemolysis induced by melittin and by a synthetic melittin analogue, Biophys. J. 37 (1982) 329–338.
[27] M.T. Tosteson, S.J. Holmes, M. Razin, D.C. Tosteson, Melittin lysis of red cells, J. Membr. Biol. 87 (1985) 35–44.
[28] S. Vunnam, P. Juvvadi, K.S. Rotondi, R.B. Merrifield, Synthesis and study of normal, enantio, retro, and retroenantio isomers of cecropin A-melittin hybrids, their end group effects and selective enzyme inactivation, J. Pept. Res. 51 (1998) 38–44.
[29] R.B. Merrifield, E.L. Merrifield, P. Juvvadi, D. Andreu, H.G. Boman, Design and synthesis of antimicrobial peptides, Ciba Found. Symp. 186 (1994) 5–20.
[30] D. Wade, A. Boman, B. Wahlin, C.M. Drain, D. Andreu, H.G. Boman, R.B. Merrifield, All-D amino acid-containing channel-forming antibiotic peptides, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 4761–4765.
[31] A. Latal, R.I. Lehrer, S. Harwig, K. Lohner, Interaction of enantiomeric protegrins with liposomes, Prog. Biophys. Mol. Biol. 65 (1996) 121.
[32] R.P. Cheng, S.H. Gellman, W.F. DeGrado, beta-Peptides: from structure to function, Chem. Rev. 101 (2001) 3219–3232.
[33] J.E. Oh, S.Y. Hong, K.H. Lee, Design, synthesis and characterization of antimicrobial pseudopeptides corresponding to membrane-active peptide, J. Pept. Res. 54 (1999) 129–136.
[34] R.I. Lehrer, A. Barton, K.A. Daher, S.S. Harwig, T. Ganz, M.E. Selsted, Interaction of human defensins with *Escherichia coli*. Mechanism of bactericidal activity, J. Clin. Invest. 84 (1989) 553–561.
[35] K. Matsuzaki, K. Sugishita, M. Harada, N. Fujii, K. Miyajima, Interactions of an antimicrobial peptide, magainin 2, with outer and inner membranes of Gram-negative bacteria, Biochim. Biophys. Acta 1327 (1997) 119–130.
[36] A. da Silva Jr., O. Teschke, Effects of the antimicrobial peptide PGLa on live *Escherichia coli*, Biochim. Biophys. Acta 1643 (2003) 95–103.
[37] T. Wieprecht, M. Dathe, R.M. Epand, M. Beyermann, E. Krause, W.L. Maloy, D.L. MacDonald, M. Bienert, Influence of the angle subtended by the positively charged helix face on the membrane activity of amphipathic, antibacterial peptides, Biochemistry 36 (1997) 12869–12880.
[38] M. Dathe, M. Schumann, T. Wieprecht, A. Winkler, M. Beyermann, E. Krause, K. Matsuzaki, O. Murase, M. Bienert, Peptide helicity and membrane surface charge modulate the balance of electrostatic and hydrophobic interactions with lipid bilayers and biological membranes, Biochemistry 35 (1996) 12612–12622.
[39] M. Sharon, Z. Oren, Y. Shai, J. Anglister, 2D-NMR and ATR-FTIR study of the structure of a cell-selective diastereomer of melittin and its orientation in phospholipids, Biochemistry 38 (1999) 15305–15316.
[40] M. Dathe, H. Nikolenko, J. Meyer, M. Beyermann, M. Bienert, Optimization of the antimicrobial activity of magainin peptides by modification of charge, FEBS Lett. 501 (2001) 146–150.
[41] T. Tachi, R.F. Epand, R.M. Epand, K. Matsuzaki, Position-dependent hydrophobicity of the antimicrobial magainin peptide affects the mode of peptide–lipid interactions and selective toxicity, Biochemistry 41 (2002) 10723–10731.
[42] K. Lohner, S.E. Blondelle, Molecular mechanisms of membrane perturbation by antimicrobial peptides and the use of biophysical studies in the design of novel peptide antibiotics, Comb. Chem. High Throughout Screen. 8 (2005) 241–256.
[43] D. Marion, M. Zasloff, A. Bax, A two-dimensional NMR study of the antimicrobial peptide magainin 2, FEBS Lett. 227 (1988) 21–26.
[44] 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.
[45] T.C. Vogt, B. Bechinger, The interactions of histidine-containing amphipathic helical peptide antibiotics with lipid bilayers. The effects of charges and pH, J. Biol. Chem. 274 (1999) 29115–29121.
[46] T. Wieprecht, M. Beyermann, J. Seelig, Binding of antibacterial magainin peptides to electrically neutral membranes: thermodynamics and structure, Biochemistry 38 (1999) 10377–10387.
[47] H.W. Huang, Action of antimicrobial peptides: two-state model, Biochemistry 39 (2000) 8347–8352.
[48] K. Matsuzaki, Molecular mechanisms of membrane perturbation by antimicrobial peptides, in: K. Lohner (Ed.), Development of Novel Antimicrobial Agents: Emerging Strategies, Horizon Scientific Press, Wymondham, Norfolk, UK, 2001, pp. 167–181.
[49] Z. Oren Y. Shai, Molecular mechanism of cell selectivity by linear amphipathic-helical and diasteriomeric antimicrobial peptides, in: K. Lohner (Ed.), Development of Novel Antimicrobial Agents: Emerging Strategies, Horizon Scientific Press, Wymondham, Norfolk, UK, 2001, pp. 183–204.
[50] M.S. Sansom, Alamethicin and related peptaibols–model ion channels, Eur. Biophys. J. 22 (1993) 105–124.
[51] K.J. Hallock, D.K. Lee, J. Omnaas, H.I. Mosberg, A. Ramamoorthy, Membrane composition determines pardaxin's mechanism of lipid bilayer disruption, Biophys. J. 83 (2002) 1004–1013.
[52] F. Porcelli, B. Buck, D.K. Lee, K.J. Hallock, A. Ramamoorthy, G. Veglia, Structure and orientation of pardaxin determined by NMR experiments in model membranes, J. Biol. Chem. 279 (2004) 45815–45823.
[53] K. Lohner, E.J. Prenner, Differential scanning calorimetry and X-ray diffraction studies of the specificity of the interaction of antimicrobial peptides with membrane-mimetic systems, Biochim. Biophys. Acta 1462 (1999) 141–156.
[54] K. Lohner, The role of membrane lipid composition in cell targeting of antimicrobial peptides, in: K. Lohner (Ed.), Development of Novel Antimicrobial Agents: Emerging Strategies, Horizon Scientific Press, Wymondham, Norfolk, UK, 2001, pp. 149–165.
[55] H. Duclohier, G. Molle, G. Spach, Antimicrobial peptide magainin I from *Xenopus* skin forms anion-permeable channels in planar lipid bilayers, Biophys. J. 56 (1989) 1017–1021.
[56] 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.
[57] B. Haimovich, J.C. Tanaka, Magainin-induced cytotoxicity in eukaryotic cells: kinetics, dose-response and channel characteristics, Biochim. Biophys. Acta 1240 (1995) 149–158.
[58] K. Matsuzaki, O. Murase, N. Fujii, K. Miyajima, An antimicrobal peptide, magainin 2, induced rapid flip-flop of phospholipids coupled with pore formation and peptide translocation, Biochemistry 35 (1996) 11361–11368.

[59] 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.
[60] L. Yang, T.A. Harroun, T.M. Weiss, L. Ding, H.W. Huang, Barrel-stave model or toroidal model? A case study on melittin pores, Biophys. J. 81 (2001) 1475–1485.
[61] B. Bechinger, Structure and function of membrane-lytic peptides, Crit. Rev. Plant Sci. 23 (2004) 271–292.
[62] Y. Pouny, D. Rapaport, A. Mor, P. Nicolas, Y. Shai, Interaction of antimicrobial dermaseptin and its fluorescently labeled analogues with phospholipid membranes, Biochemistry 31 (1992) 12416–12423.
[63] B. Bechinger, K. Shon, H. Eck, M. Zasloff, S.J. Opella, NMR studies of magainin peptide antibiotics in membranes, Hoppe-Seyler Z. Physiol. Chem. 371 (1990) 758.
[64] 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.
[65] Z. Oren, Y. Shai, Mode of action of linear amphipathic alpha-helical antimicrobial peptides, Biopolymers 47 (1998) 451–463.
[66] H. Steiner, D. Andreu, R.B. Merrifield, Binding and action of cecropin and cecropin analogues: antibacterial peptides from insects, Biochim. Biophys. Acta 939 (1988) 260–266.
[67] D. Juretic, R.W. Hendler, F. Kamp, W.S. Caughey, M. Zasloff, H.V. Westerhoff, Magainin oligomers reversibly dissipate delta microH+ in cytochrome oxidase liposomes, Biochemistry 33 (1994) 4562–4570.
[68] B. Christensen, J. Fink, R.B. Merrifield, D. Mauzerall, Channel-forming properties of cecropins and related model compounds incorporated into planar lipid membranes, Proc. Natl. Acad. Sci. U. S. A. 85 (1988) 5072–5076.
[69] B. Bechinger, The structure, dynamics and orientation of antimicrobial peptides by multidimensional solid-state NMR spectroscopy, Biochim. Biophys. Acta 1462 (1999) 157–183.
[70] B. Bechinger, Detergent-like properties of magainin antibiotic peptides: a ^{31}P solid-state NMR spectroscopy study, Biochim. Biophys. Acta 1712 (2005) 101–108.
[71] K.J. Hallock, D.K. Lee, A. Ramamoorthy, MSI-78, an analogue of the magainin antimicrobial peptides, disrupts lipid bilayer structure via positive curvature strain, Biophys. J. 84 (2003) 3052–3060.
[72] K. Lohner, Effects of small organic molecules on phospholipid phase transitions, Chem. Phys. Lipids 57 (1991) 341–362.
[73] T.D. Madden, P.R. Cullis, Stabilization of bilayer structure for unsaturated phosphatidylethanolamines by detergents, Biochim. Biophys. Acta 684 (1982) 149–153.
[74] K. Müller, G. LIPKA, K. Lohner, P. Laggner, The effect of the detergent cetyltrimethaylammoniumchloride on the phase behavior of Dipalmitoylphosphatidylcholine. A high precision calorimetric studies, Thermochim. Acta 94 (1985) 187–197.
[75] A.M. Christensen, J. Schaefer, Solid-state NMR determination of intra- and intermolecular 31P-13C distances for shikimate 3-phosphate and [1−13C]glyphosate bound to enolpyruvylshikimate-3-phosphate synthase, Biochemistry 32 (1993) 2868–2873.
[76] P. Schlieper, E. De Robertis, Triton X-100 as a channel-forming substance in artificial lipid bilayer membranes, Arch. Biochem. Biophys. 184 (1977) 204–208.
[77] G.M. Alder, W.M. Arnold, C.L. Bashford, A.F. Drake, C.A. Pasternak, U. Zimmermann, Divalent cation-sensitive pores formed by natural and synthetic melittin and by Triton X-100, Biochim. Biophys. Acta 1061 (1991) 111–120.
[78] V.F. Antonov, V.V. Petrov, A.A. Molnar, D.A. Predvoditelev, A.S. Ivanov, The appearance of single-ion channels in unmodified lipid bilayer membranes at the phase transition temperature, Nature 283 (1980) 585–586.
[79] K. Kaufmann, I. Silman, The induction by protons of ion channels through lipid bilayer membranes, Biophys. Chem. 18 (1983) 89–99.
[80] K. Yoshikawa, T. Fujimoto, T. Shimooka, H. Terada, N. Kumazawa, T. Ishii, Electrical oscillation and fluctuation in phospholipid membranes. Phospholipids can form a channel without protein, Biophys. Chem. 29 (1988) 293–299.
[81] D.J. Woodbury, Pure lipid vesicles can induce channel-like conductances in planar bilayers, J. Membr. Biol. 109 (1989) 145–150.
[82] M. Miteva, M. Andersson, A. Karshikoff, G. Otting, Molecular electroporation: a unifying concept for the description of membrane pore formation by antibacterial peptides, exemplified with NK-lysin, FEBS Lett. 462 (1999) 155–158.
[83] T.C. Terwilliger, D. Eisenberg, The structure of melittin: I. Structure determination and partial refinement, J. Biol. Chem. 257 (1982) 6010–6015.
[84] J.E. Fitton, Physicochemical studies on delta Haemolysin, a Staphylococcal cytolytic polypeptide, FEBS Lett. 130 (1981) 257–260.
[85] E. Thiaudiere, O. Siffert, J.-C. Talbot, J. Bolard, J.E. Alouf, J. Dufourcq, The amphiphilic a-helix concept. Consequences on the structure of staphylococcal d-toxin in solution and bound to lipids, Eur. J. Biochem. 195 (1991) 203–213.
[86] Z. Oren, Y. Shai, Cyclization of a cytolytic amphipathic alpha-helical peptide and its diastereomer: effect on structure, interaction with model membranes, and biological function, Biochemistry 39 (2000) 6103–6114.
[87] I. Kustanovich, D.E. Shalev, M. Mikhlin, L. Gaidukov, A. Mor, Structural requirements for potent versus selective cytotoxicity for antimicrobial dermaseptin S4 derivatives, J. Biol. Chem. 277 (2002) 16941–16951.
[88] L. Stella, C. Mazzuca, M. Venanzi, A. Palleschi, M. Didone, F. Formaggio, C. Toniolo, B. Pispisa, Aggregation and water–membrane partition as major determinants of the activity of the antibiotic peptide trichogin GA IV, Biophys. J. 86 (2004) 936–945.
[89] R.E. Hancock, D.S. Chapple, Peptide antibiotics, Antimicrob. Agents Chemother. 43 (1999) 1317–1323.
[90] C. Auvin-Guette, S. Rebufatt, Y. Prigent, B. Bodo, Trichogin A IV, an 11-residue lipopeptaibol from *Trichoderma longibrachiatum*, J. Am. Chem. Soc. 114 (1992) 2170–2174.
[91] I.S. Radzishevsky, S. Rotem, F. Zaknoon, L. Gaidukov, A. Dagan, A. Mor, Effects of acyl versus aminoacyl conjugation on the properties of antimicrobial peptides, Antimicrob. Agents Chemother. 49 (2005) 2412–2420.
[92] N. Sal-Man, Z. Oren, Y. Shai, Preassembly of membrane-active peptides is an important factor in their selectivity toward target cells, Biochemistry 41 (2002) 11921–11930.
[93] E. Grant Jr., T.J. Beeler, K.M. Taylor, K. Gable, M.A. Roseman, Mechanism of magainin 2a induced permeabilization of phospholipid vesicles, Biochemistry 31 (1992) 9912–9918.
[94] I.V. Polozov, G.M. Anantharamaiah, J.P. Segrest, R.M. Epand, Osmotically induced membrane tension modulates membrane permeabilization by class L amphipathic helical peptides: nucleation model of defect formation, Biophys. J. 81 (2001) 949–959.
[95] M. Vaara, T. Vaara, Ability of cecropin B to penetrate the enterobacterial outer membrane, Antimicrob. Agents Chemother. 38 (1994) 2498–2501.
[96] J.N. Israelachvili, S. Marcelja, R.G. Horn, Physical principles of membrane organization, Q. Rev. Biophys. 13 (1980) 121–200.
[97] J. Gallova, F. Devinsky, P. Balgavy, Interaction of surfactants with model and biological membranes: II. Effect of N-alkyl-N,N,N-trimethylammonium ions on phosphatidylcholine bilayers as studied by spin probe ESR, Chem. Phys. Lipids 53 (1990) 231–241.
[98] 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.
[99] J. Karlovska, K. Lohner, G. Degovics, I. Lacko, F. Devinsky, P. Balgavy, Effects of non-ionic surfactants N-alkyl-N,N-dimethylamine-N-oxides on the structure of a phospholipid bilayer: small-angle X-ray diffraction study, Chem. Phys. Lipids 129 (2004) 31–41.
[100] P. Balgavy, F. Devinsky, Cut-off effects in biological activities of surfactants, Adv. Colloid Interface Sci. 66 (1996) 23–63.
[101] R.E. Glover, R.R. Smith, M.V. Jones, S.K. Jackson, C.C. Rowlands, An EPR investigation of surfactant action on bacterial membranes, FEMS Microbiol. Lett. 177 (1999) 57–62.
[102] P.R. Cullis, B. de Kruijff, Lipid polymorphism and the functional roles of lipids in biological membranes, Biochim. Biophys. Acta 559 (1979) 399–420.
[103] K. Matsuzaki, K. 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.
[104] A. Colotto, D.P. Kharakoz, K. Lohner, P. Laggner, Ultrasonic study of melittin effects on phospholipid model membranes, Biophys. J. 65 (1993) 2360–2367.
[105] R. Smith, F. Separovic, T.J. Milne, A. Whittaker, F.M. Bennett, B.A. Cornell, A. Makriyannis, Structure and orientation of the pore-forming peptide, melittin, in lipid bilayers, J. Mol. Biol. 241 (1994) 456–466.
[106] A.S. Ladokhin, S.H. White, 'Detergent-like' permeabilization of anionic lipid vesicles by melittin, Biochim. Biophys. Acta 1514 (2001) 253–260.
[107] M. Posch, U. Rakusch, C. Mollay, P. Laggner, Cooperative effects in the interaction between melittin and phosphatidylcholine model membranes. Studies by temperature scanning densitometry, J. Biol. Chem. 258 (1983) 1761–1766.
[108] A. Collotto, K. Lohner, P. Laggner, Low-dose effects of melittin on phospholipid structure. Differences between diester and diether phospholipids, Prog. Colloid Polym. Sci. 89 (1992) 334.
[109] P.F. Almeida, W.L. Vaz, T.E. Thompson, Percolation and diffusion in three-component lipid bilayers: effect of cholesterol on an equimolar mixture of two phosphatidylcholines, Biophys. J. 64 (1993) 399–412.
[110] J. Dufourcq, J.F. Faucon, G. Fourche, J.L. Dasseux, M. le Maire, T. Gulik-Krzywicki, Morphological changes of phosphatidylcholine bilayers induced by melittin: vesicularization, fusion, discoidal particles, Biochim. Biophys. Acta 859 (1986) 33–48.
[111] M. Monette, M. Lafleur, Modulation of melittin-induced lysis by surface charge density of membranes, Biophys. J. 68 (1995) 187–195.
[112] E.J. Dufourc, I.C. Smith, J. Dufourcq, Molecular details of melittin-induced lysis of phospholipid membranes as revealed by deuterium and phosphorus NMR, Biochemistry 25 (1986) 6448–6455.
[113] C.E. Dempsey, A. Watts, A deuterium and phosphorus-31 nuclear magnetic resonance study of the interaction of melittin with dimyristoylphosphatidylcholine bilayers and the effects of contaminating phospholipase A2, Biochemistry 26 (1987) 5803–5811.
[114] J.F. Faucon, J.M. Bonmatin, J. Dufourcq, E.J. Dufourc, Acyl chain length dependence in the stability of melittin-phosphatidylcholine complexes. A light scattering and 31P-NMR study, Biochim. Biophys. Acta 1234 (1995) 235–243.
[115] K. Lohner, P. Laggner, J.H. Freer, Dilatometric and calorimetric studies of the effect of *Staphylococcus aureus* delta-lysin on the phospholipid phase transition, J. Solution Chem. 15 (1986) 189–198.
[116] K. Lohner, E. Staudegger, E.J. Prenner, R.N. Lewis, M. Kriechbaum, G. Degovics, R.N. McElhaney, Effect of staphylococcal delta-lysin on the thermotropic phase behavior and vesicle morphology of dimyristoylphosphatidylcholine lipid bilayer model membranes. Differential scanning calorimetric, 31P nuclear magnetic resonance and Fourier transform infrared spectroscopic, and X-ray diffraction studies, Biochemistry 38 (1999) 16514–16528.
[117] M. Thelestam, in: C.S.F. Easman, C.A. Easman (Eds.), Staphylococci and Staphylococcal Diseases, Academic Press, London, 1983, pp. 705–744.
[118] I.R. Mellor, D.H. Thomas, M.S. Sansom, Properties of ion channels formed by *Staphylococcus aureus* delta-toxin, Biochim. Biophys. Acta 942 (1988) 280–294.
[119] A.M. Batenburg, J.H. van Esch, B. de Kruijff, Melittin-induced changes of the macroscopic structure of phosphatidylethanolamines, Biochemistry 27 (1988) 2324–2331.
[120] A.M. Batenburg, J.C. Hibbeln, A.J. Verkleij, B. de Kruijff, Melittin induces HII phase formation in cardiolipin model membranes, Biochim. Biophys. Acta 903 (1987) 142–154.
[121] A.M. Batenburg, J.H. van Esch, J. Leunissen-Bijvelt, A.J. Verkleij, B. de Kruijff, Interaction of melittin with negatively charged phospholipids: consequences for lipid organization, FEBS Lett. 223 (1987) 148–154.
[122] A.M. Batenburg, J.C. Hibbeln, B. de Kruijff, Lipid specific penetration of melittin into phospholipid model membranes, Biochim. Biophys. Acta 903 (1987) 155–165.
[123] C. Dempsey, M. Bitbol, A. Watts, Interaction of melittin with mixed phospholipid membranes composed of dimyristoylphosphatidylcholine and dimyristoylphosphatidylserine studied by deuterium NMR, Biochemistry 28 (1989) 6590–6596.
[124] S. Ohki, E. Marcus, D.K. Sukumaran, K. Arnold, Interaction of melittin with lipid membranes, Biochim. Biophys. Acta 1194 (1994) 223–232.
[125] F. Porcelli, B.A. Buck-Koehntop, S. Thennarasu, A. Ramamoorthy, G. Veglia, Structures of the dimeric and monomeric variants of magainin antimicrobial peptides (MSI-78 and MSI-594) in micelles and bilayers, determined by NMR spectroscopy, Biochemistry 45 (2006) 5793–5799.
[126] 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.
[127] S. Ludtke, K. He, H. Huang, Membrane thinning caused by magainin 2, Biochemistry 34 (1995) 16764–16769.
[128] C. Münster, Y. Lu, S. Schinzel, B. Bechinger, T. Salditt, Grazing incidence X-ray scattering of highly aligned phospholipid membranes containing antimicrobial peptides magainin 2, Eur. Biophys. J. 28 (1999) 683–688.
[129] F.Y. Chen, M.T. Lee, H.W. Huang, Evidence for membrane thinning effect as the mechanism for peptide-induced pore formation, Biophys. J. 84 (2003) 3751–3758.
[130] A. Mecke, D.K. Lee, A. Ramamoorthy, B.G. Orr, M.M. Banaszak Holl, Membrane thinning due to antimicrobial peptide binding: an atomic force microscopy study of MSI-78 in lipid bilayers, Biophys. J. 89 (2005) 4043–4050.
[131] 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.
[132] S. Dvinskikh, U. Durr, K. Yamamoto, A. Ramamoorthy, A high-resolution solid-state NMR approach for the structural studies of bicelles, J. Am. Chem. Soc. 128 (2006) 6326–6327.
[133] A.J. Mason, A. Matinez, C. Glaubitz, O. Danos, A. Kichler, B. Bechinger, The antibiotic and DNA-transfecting peptide LAH4 selectively associates with, and disorders, anionic lipids in mixed membranes, FASEB J. 20 (2006) 320–322.
[134] A.J. Mason, I.N. Husnal-Chotimah, P. Bertani, B. Bechinger, A spectroscopic study of the membrane interaction of the antimicrobial peptide pleurocidin, Mol. Membr. Biol. 23 (2006) 185–194.
[135] K. Lohner, A. Latal, R.I. Lehrer, T. Ganz, Differential scanning microcalorimetry indicates that human defensin, HNP-2, interacts specifically with biomembrane mimetic systems, Biochemistry 36 (1997) 1525–1531.
[136] B.B. Bonev, W.C. Chan, B.W. Bycroft, G.C. Roberts, A. Watts, Interaction of the lantibiotic nisin with mixed lipid bilayers: a 31P and 2H NMR study, Biochemistry 39 (2000) 11425–11433.
[137] A. Latal, G. Degovics, R.F. Epand, R.M. Epand, K. Lohner, Structural aspects of the interaction of peptidyl–glycylleucine–carboxyamide, a highly potent antimicrobial peptide from frog skin, with lipids, Eur. J. Biochem. 248 (1997) 938–946.
[138] S. Kobayashi, K. Takeshima, C.B. Park, S.C. Kim, K. Matsuzaki, Interactions of the novel antimicrobial peptide buforin 2 with lipid bilayers: proline as a translocation promoting factor, Biochemistry 39 (2000) 8648–8654.
[139] T. Nakamura, H. Furunaka, T. Miyata, F. Tokunaga, T. Muta, S. Iwanaga, M. Niwa, T. Takao, Y. Shimonishi, Tachyplesin, a class of antimicrobial peptide from the hemocytes of the horseshoe crab (*Tachypleus tridentatus*), isolation and chemical structure, J. Biol. Chem. 263 (1988) 16709–16713.
[140] D. Gidalevitz, Y. Ishitsuka, A.S. Muresan, O. Konovalov, A.J. Waring, R. I. Lehrer, K.Y. Lee, Interaction of antimicrobial peptide protegrin with biomembranes, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 6302–6307.
[141] E.J. Prenner, R.N. Lewis, L.H. Kondejewski, R.S. Hodges, R.N. McElhaney, Differential scanning calorimetric study of the effect of the antimicrobial peptide gramicidin S on the thermotropic phase behavior of phosphatidylcholine, phosphatidylethanolamine and phosphatidylglycerol lipid bilayer membranes, Biochim. Biophys. Acta 1417 (1999) 211–223.
[142] P.M. Abuja, A. Zenz, M. Trabi, D.J. Craik, K. Lohner, The cyclic antimicrobial peptide RTD-1 induces stabilized lipid–peptide domains

more efficiently than its open-chain analogue, FEBS Lett. 566 (2004) 301–306.
[143] R.A. Demel, T. Peelen, R.J. Siezen, B. de Kruijff, O.P. Kuipers, Nisin Z, mutant nisin Z and lacticin 481 interactions with anionic lipids correlate with antimicrobial activity. A monolayer study, Eur. J. Biochem. 235 (1996) 267–274.
[144] M.A. Carbone, P.M. Macdonald, Cardiotoxin II segregates phosphatidylglycerol from mixtures with phosphatidylcholine: (31)P and (2)H NMR spectroscopic evidence, Biochemistry 35 (1996) 3368–3378.
[145] R.M. Epand, Lipid polymorphism and protein–lipid interactions, Biochim. Biophys. Acta 1376 (1998) 353–368.
[146] I.R. Mellor, M.S. Sansom, Ion-channel properties of mastoparan, a 14-residue peptide from wasp venom, and of MP3, a 12-residue analogue, Proc. R. Soc. London, B Biol. Sci. 239 (1990) 383–400.
[147] E.L. Merrifield, S.A. Mitchell, J. Ubach, H.G. Boman, D. Andreu, R.B. Merrifield, D-enantiomers of 15-residue cecropin A-melittin hybrids, Int. J. Pept. Protein Res. 46 (1995) 214–220.
[148] J.D. Lear, Z.R. Wasserman, W.F. DeGrado, Synthetic amphiphilic peptide models for protein ion channels, Science 240 (1988) 1177–1181.
[149] K. Anzai, M. Hamasuna, H. Kadono, S. Lee, H. Aoyagi, Y. Kirino, Formation of ion channels in planar lipid bilayer membranes by synthetic basic peptides, Biochim. Biophys. Acta 1064 (1991) 256–266.
[150] Y. Higashimoto, H. Kodama, M. Jelokhani-Niaraki, F. Kato, M. Kondo, Structure–function relationship of model Aib-containing peptides as ion transfer intermembrane templates, J. Biochem. (Tokyo) 125 (1999) 705–712.
[151] S.E. Blondelle, R.A. Houghten, Design of model amphipathic peptides having potent antimicrobial activities, Biochemistry 31 (1992) 12688–12694.
[152] K.H. Lee, Development of short antimicrobial peptides derived from host defense peptides or by combinatorial libraries, Curr. Pharm. Des. 8 (2002) 795–813.
[153] L. Beven, S. Castano, J. Dufourcq, A. Wieslander, H. Wroblewski, The antibiotic activity of cationic linear amphipathic peptides: lessons from the action of leucine/lysine copolymers on bacteria of the class Mollicutes, Eur. J. Biochem. 270 (2003) 2207–2217.
[154] H. Schroder-Borm, R. Willumeit, K. Brandenburg, J. Andra, Molecular basis for membrane selectivity of NK-2, a potent peptide antibiotic derived from NK-lysin, Biochim. Biophys. Acta 1612 (2003) 164–171.
[155] Y. Nakajima, X.M. Qu, S. Natori, Interaction between liposomes and sarcotoxin IA, a potent antibacterial protein of *Sarcophaga peregrina* (flesh fly), J. Biol. Chem. 262 (1987) 1665–1669.
[156] E.M. Tytler, G.M. Anantharamaiah, D.E. Walker, V.K. Mishra, M.N. Palgunachari, J.P. Segrest, Molecular basis for prokaryotic specificity of magainin-induced lysis, Biochemistry 34 (1995) 4393–4401.
[157] A. Pokorny, P.F. Almeida, Permeabilization of raft-containing lipid vesicles by delta-lysin: a mechanism for cell sensitivity to cytotoxic peptides, Biochemistry 44 (2005) 9538–9544.
[158] P.F. Almeida, F.W. Wiegel, A simple theory of peptide interactions on a membrane surface: excluded volume and entropic order, J. Theor. Biol. 238 (2006) 269–278.