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Review

The impact of peptides on lipid membranes

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ABSTRACT

We review the fundamental strategies used by small peptides to associate with lipid membranes and how the different strategies impact on the structure and dynamics of the lipids. In particular we focus on the binding of amphiphilic peptides by electrostatic and hydrophobic forces, on the anchoring of peptides to the bilayer by acylation and prenylation, and on the incorporation of small peptides that form well-defined channels. The effect of lipid–peptide interactions on the lipids is characterized in terms of lipid acyl-chain order, membrane thickness, membrane elasticity, permeability, lipid-domain and annulus formation, as well as acyl-chain dynamics. The different situations are illustrated by specific cases for which experimental observations can be interpreted and supplemented by theoretical modeling and simulations. A comparison is made with the effect on lipids of trans-membrane proteins. The various cases are discussed in the context of the possible roles played by lipid–peptide interactions for the biological, physiological, and pharmacological function of peptides.

Contents

1. Introduction

The interplay between lipids and peptides/proteins has remained an elusive problem in membrane science for a long time and there still a number of outstanding major questions to be resolved [1–4]. Firstly, it remains a mystery why Nature has embarked on a strategy involving the use of a large diversity of lipids for each type of cellular membrane and how this diversity impacts on and supports protein function and cellular signaling. Until recently, little was known regarding the actual composition of membranes in terms of lipid species, but at the moment there is an upsurge in the available data for the exact lipid composition of specific membranes and organelles, mainly obtained by the use of

Abbreviations: AMP, antimicrobial peptides; CD, circular dichroism; DiPhPC, diphytanolphosphatidylcholine; DMPC, dimyristoyl phosphatidylcholine; DMPG, dimyristoyl phosphatidylglycerol; DOPE, 16:0/18:1c9-palmitoyloleyl phosphatidylethanolamine; DPC, dodecylphosphocholine; DPPC, dipalmitoyl phosphatidylcholine; gA, gramicidin A; GMO, glycerol 1-monooleate; MAG, magainin; NMR, nuclear magnetic resonance; OVIS, ovispirin-1; MD, Molecular Dynamics; PC, phosphatidylcholine; PG, phosphatidylglycerol; PS, phosphatidylserine; POPC, 16:0/18:1c9 palmitoyloleyl phosphatidylcholine; SDS, sodiumdodecylsulfate; TFE, tri-fluoro-ethanol

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modern lipidomics techniques. Secondly, it appears that in many cases the lipids are more impressed by the proteins than visa versa. Upon association of peptides and proteins with membranes, structural and dynamical properties of the lipids are often strongly affected on different length and time scales. Peptide and protein structure seems often only to be little affected by the lipids. Still, subtle but very fundamental properties of the lipid bilayers, e.g. the lateral pressure profile [5], may indeed couple to integral membrane function, and the details of the structure and dynamics of small amphiphilic peptides bound to membrane interfaces are often controlled by the specific interaction with the different parts of the lipid bilayer. The structure of antimicrobial peptides is also delicately modulated by lipid bilayers.

In the present paper we shall focus on the effect of mostly small peptides on lipid-bilayer properties. Many of these peptides also exert biological effects on biological membranes, such as antimicrobial, antifungal, antiviral, or anticancer activity. In some cases these peptides act in synergy with other drugs to enhance their pharmacological or physiological effects [6]. These effects are in many cases mediated by structural changes in the lipid bilayer. In order to unravel the mechanism of action it is important to study and characterize in detail the various modulating effects on the lipids caused by the interaction with the peptides.

A very large number of peptides have been studied with an impressive range of experimental methodologies and in some cases the experimental studies have been paralleled by theoretical considerations involving models and simulations of models. We shall in the present paper focus on a small selection of such studies where experiments and atomic-scale Molecular Dynamics (MD) simulations together have provided an enhanced understanding of the different ways peptides modulate lipid membranes. Although we will not describe in detail how the lipids modulate peptides and proteins, we shall for a few selected cases provide some information on lipid-modulated protein and membrane function.

2. Modes of lipid–peptide interactions

Peptides that associate with membranes are drawn to the lipid bilayer by different mechanisms. Most often, the peptide has some amphiphilic character and the hydrophobic effect acts as to allocate the peptide in the hydrophilic–hydrophobic interfacial layer of the membrane. Additionally, electrostatic effects may be in charge. Upon association with the membrane, the peptides often change their conformation [7]. In other cases, even for strongly hydrophilic and water-soluble peptides, the binding to the bilayer can be facilitated by hydrophobic anchors, such as acylation or prenylation. These modes of association can have a strong impact on the lipids, but in some cases the effect is not very pronounced and even subtle and difficult to unravel. The various modes of the interaction of peptides with lipid bilayers are illustrated in Fig. 1. Upon association, binding, and possible penetration of the bilayer, some peptides tend to form oligomers that may span the membrane or form aggregates that constitute patches or even pores in the bilayer. In such cases the effects on the bilayer properties are very pronounced and may even lead to destabilization of the bilayer.

3. Amphiphilic antimicrobial peptides

3.1. Overview

The amphiphilic antimicrobial peptides (AMP) form a large class of small (10–40 amino acids), amphipathic, and often positively charged peptides. These peptides have a drastic impact on the structure and integrity of both natural membranes in cells, and of artificial membranes in vitro. AMPs are synthesized by most living organisms for immunological protection against invading pathogens [8] and are capable of efficiently killing bacteria and fungi. There is sufficient evidence that the primary target of AMPs is the lipid matrix of the plasma membrane of cells [8]. AMPs kill pathogenic cells by perturbation of the plasma membrane and subsequent cell lysis [9]. AMPs also have auxiliary functions, and are key components in several immunological signaling pathways which recruit immune machinery near infection sites [10]. Here, we will only focus on the lipid bilayer perturbing properties of AMPs.

The potential application of AMPs as novel therapeutic antibiotics fuelled an enormous body of research. Hundreds of artificial AMPs have been synthesized, characterized and investigated by biochemical and biophysical means, both theoretical and experimental. We will attempt to provide an overview of the general mechanism of peptide-mediated membrane perturbation by focusing on a few exemplary peptides for which experiments have been complemented by theory and molecular simulations. Specifically, we will focus on the helical AMPs magainin [11] and ovispirin-1 [12]. Magainin will be used as an example to describe the general lipid-bilayer perturbation mechanism of AMPs. Ovispirin-1 will be used to explain the selectivity of AMPs towards membranes of specific compositions.

3.2. Magainin

Magainin (MAG) is a 23-amino acid cationic AMP produced by the African frog *Xenopus laevis*. MAG has antibacterial, antifungal, antiviral and anticancer activity. It has been shown that the biological activity of MAG correlates with its induced leakage of lipid vesicles [13], confirming its bilayer-perturbation mechanism of action. The sequence of the MAG peptide is GIGKF LHSAK KFGKA FVGEI MNS. The peptide carries a net charge of +3 at neutral pH, and has a several hydrophobic residues. MAG folds into an amphipathic helix in the presence of organic solvents, detergents [14] or a membrane interface [15–17]. The properties of small size, cationic charge and a helical amphipathic structure assist quick diffusion to the membrane interface, electrostatic binding to the lipid head groups, and subsequent perturbation of membrane structure by hydrophobic association and/or a detergent-like action once a sufficient number of peptides aggregate locally.

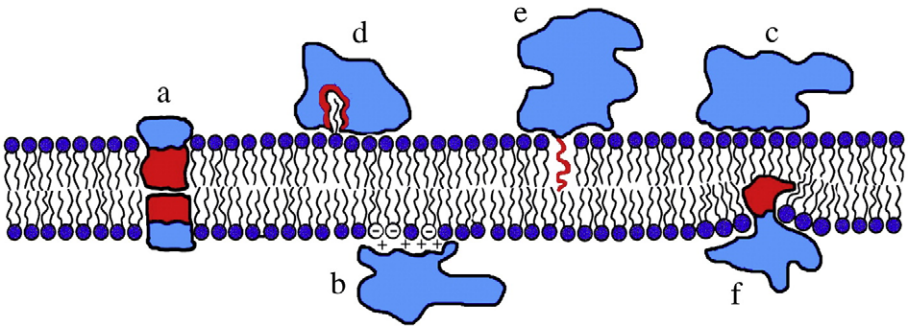

Fig. 1. Schematic illustration of the different strategies used by peptides to bind to, anchor to, imbed in, and penetrate lipid membranes. (a) trans-membrane-spanning amphiphilic peptide dimer; (b) electrostatic binding; (c) non-specific binding by weak physical forces; (d) anchoring via a lipid extended conformation; (e) anchoring by an acyl-chain anchor attached to the protein; (f) amphiphilic peptide partially penetrating the bilayer.

Numerous mutants of MAG have been designed to maximize the antimicrobial efficacy of the peptide. Here, we will refer to a few typical studies which show that positive charge and amphiphilicity are necessary for the antimicrobial action of MAG. The MSI-78 analogue of MAG (GIGKF LKKAK KFGKA FVKIL KK-NH2), has an increased positive charge (+9) and higher antimicrobial activity compared to MAG. Addition of a 10-lysine residue tail to MAG improved antimicrobial activity without affecting hemolytic activity (toxicity against red blood cells) [18]. Increasing the charge systematically from 0 to +6 in magainin analogues increased the strength of binding to acidic phospholipids. However, increasing charge beyond +5 increased hemolytic activity [19]. Helix-promoting mutations like substitution of glycine residues by alanine residues drastically increased both antibacterial and hemolytic activity [20]. Conversely, mutants with helix disrupting mutations like introduction of D-amino acids usually had reduced permeabilizing influence on lipid vesicles [21]. Mutations with increased hydrophobicity increased the binding affinity to small neutral and zwitterionic unilamellar vesicles [22]. Similarly, mutants with increased hydrophobicity increased the extent of calcein leakage from large unilamellar vesicles (LUVs), while also increasing hemolytic activity [23].

To summarize, although hydrophobic residues are essential for antimicrobial activity, excessive hydrophobicity increases binding affinity to zwitterionic membranes, and consequently hemolytic activity. Secondly, higher positive charge increases antimicrobial properties, but very high charges make the peptides hemolytic as well. It is important to note that the functional properties of different types of AMPs do not always strictly conform to these rules. The presence of a minimal number of charged and hydrophobic residues is required for antimicrobial activity. However, the **optimal** number of such residues for maximum antimicrobial property and minimum cytotoxicity (toxicity towards white blood cells) varies widely for different peptides, even within the same structural type.

Recently, studies have investigated the effect of MAG on the mechanical properties of membranes, in particular the measurement of bending rigidity of GUVs [24]. These studies show a general trend of reducing the bending rigidity significantly at very low peptides levels in the membrane (<1% surface area coverage). This suggests that the mechanical integrity of the membrane is strongly affected even at minute peptide levels. It was further possible to demonstrate that the peptides have some affinity across the monolayer leaflets of the bilayer [24], which could serve the basis for transient pre-pores, defect structures making the membrane leaky [25].

3.3. Pore models

Different models of pores through the membranes have been popular, like the trans-membrane helical bundles [26,27], the toroidal model [28,29], the carpet model [30] and the detergent-like peptide model [31,32], for review see [33]. It has been challenging to prove that antimicrobial action is directly linked to any of these models. Further, the matter gets complicated because the models are not mutually exclusive and may be applicable for the same peptide under different system conditions. The only experimental evidence of molecular structure of these pores comes from low-resolution neutron and X-ray diffraction studies. Such experiments can detect differences in pore sizes induced in bilayers upon binding of different AMPs. The number of peptides comprising a single pore can also be approximately estimated [13]. Computer simulations of peptide-membrane systems are an attractive alternative to access finer molecular details, but are limited by the time and length scales accessible.

3.4. Molecular dynamics simulations of magainin in lipid bilayers

Due to limitations in computational power, atomistic MD simulations of peptides are not able to simultaneously access time scales of microseconds to milliseconds, and length scales of microns which are required to investigate the binding, aggregation and perturbation of the lipid membrane. However, it is possible to look at these events in isolation by careful modeling of the initial simulation setup. For example, the binding modes of single peptides near lipid interfaces can be examined by placing a single peptide very close to the interface in various conformations, and running short 10–100 ns simulations. Similarly, the molecular details of the pore-forming mechanism of AMPs can be investigated by placing several AMPs close to each other, and close to the membrane interface, and running the simulation for 50–250 ns. We will describe one simulation of each type.

From MD simulations of a single magainin and MSI-78 monomer near a POPC interface, it was concluded that the binding of peptides to interfaces was mediated by lysine residues which formed H-bonds with either the phosphate oxygen atoms or the glycerol oxygen atoms on the lipid head groups [34]. Similar H-bonding has been reported for other peptides in simulations (Fig. 2)[25,35–43]. The peptide altered several structural and dynamic properties of the membrane. Firstly, the insertion of the peptide into the bilayer lowered the order parameters of the lipids locally bound to the peptide. However, the hydrocarbon chains of the lipids that were far from the peptide were not affected. Thus, on average, the peptide had only a slight disordering effect on the hydrocarbon tails of the membrane. Second, the peptides caused a local thinning effect by inducing positive curvature strain on the lipids. The thinning effect was caused by interaction of charged residues with lipid head groups. Similar conclusions with respect to the order parameters and local thinning were drawn from simulations of β-sheet peptides in a DLPC lipid bilayer (Fig. 3) [36].

In more recent work, Marrink and coworkers [25] were able to demonstrate, for the first time using atomistic simulations, the spontaneous formation of a pore-like defect in a ~7×7 nm patch of a DPPC membrane. The pore was induced by the presence of multiple AMPs near the interface. One, two, or four peptides were placed near the membrane in separate simulations. Spontaneous “pore” or defect

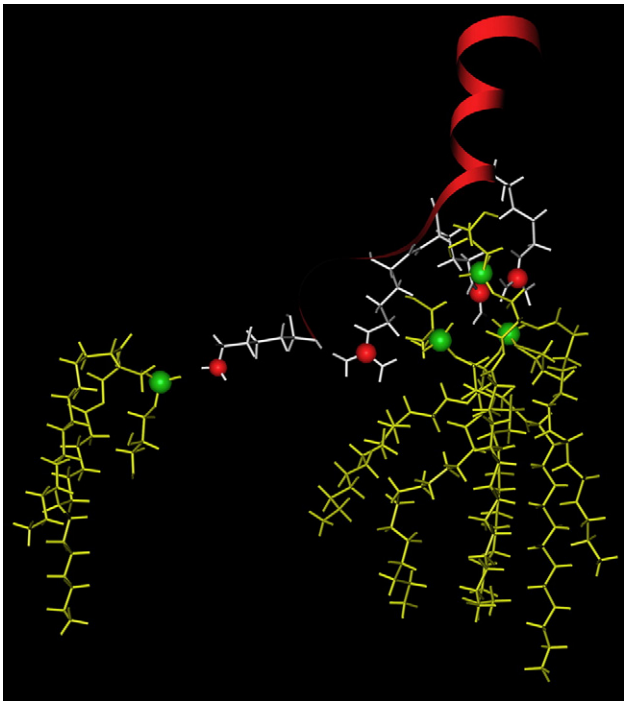

Fig. 2. Simulation snapshot of H-bonding between a helical peptide (ovispirin-1) and DMPG lipids (unpublished data). Only few lipids are shown for clarity, and are colored yellow. The phosphate groups are shown as green spheres, the positively charged side-chain centers of cationic peptide residues are shown as red spheres and the peptide side chains are shown in white. The negatively charged phosphate oxygen atoms form strong H-bonds with the positively charged side chains. Such interactions are frequently observed in MD simulations of peptide-bilayer systems (see text).

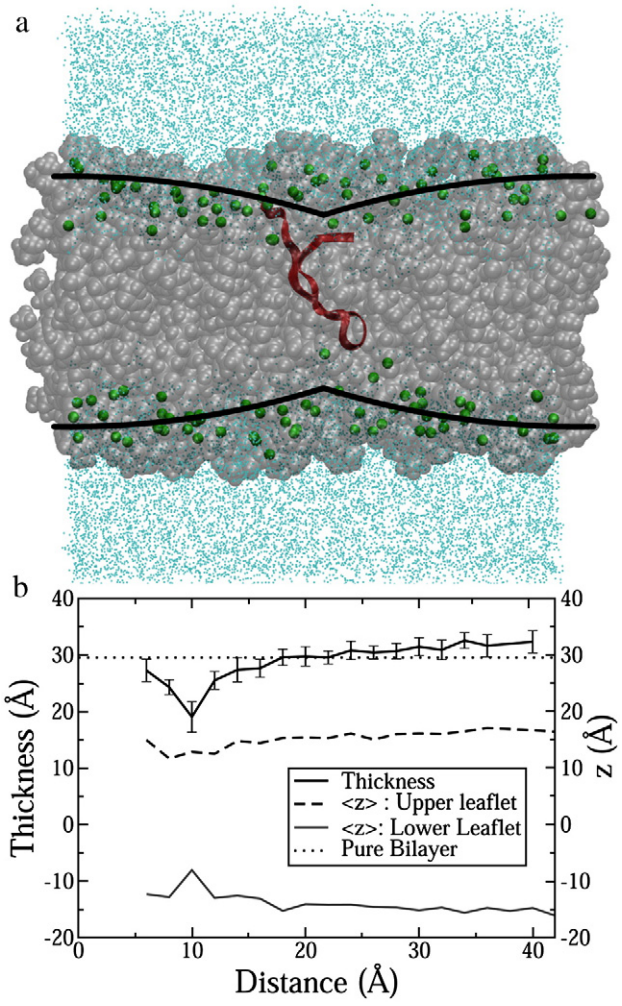

Fig. 3. (a) Simulation snapshot and a schematic example of positive curvature strain induced in a lipid bilayer by a membrane-spanning peptide: Protegrin-1 [36]. (b) Bilayer thickness (phosphate-to-phosphate distance) as a function of the distance from the center of mass of the peptide is plotted. The average *Z* coordinate of the upper and lower leaflets has also been shown as a function of the distance from the closest terminus of the peptide. Only the local bilayer thickness is altered. Adapted from Ref. [36].

formation was observed when the number of peptides placed near the membrane was increased to four, but only when the peptides aggregated. Thus, like in experiments, the simulations show that the pore-formation process is concentration dependent. Note that a direct quantitative comparison of the peptide:lipid ratio between simulations and experiments is not possible because periodic boundary conditions are used in simulations. In agreement with theoretical models [8], the pore was lined with hydrophilic lipid headgroups. The authors [25] described the pore as a "disordered toroidal pore" because unlike the theoretical model, only a single peptide was found in the center of the pore. However, this was probably the result of the presence of an insufficient number of peptides in the simulation. It is possible that if a larger number of peptides are placed near a membrane patch, the toroidal pore formed might be stabilized with the presence of a larger number of peptides lining the pore. When the peptides aggregate, they cause a local expansion of the area of the bilayer by about 3% leading to a compressive stress in the bound lipid monolayer and a corresponding expansive stress in the other monolayer. Furthermore, the local width of the phosphate distribution along the bilayer normal also increased by ~40%. The presence of the peptide thus disordered the lipids in its vicinity. On average, there was no significant effect on lipid order parameters or on the thickness of the bilayer.

3.5. Selectivity of AMPs for anionic membranes

For simplicity's sake, in this section, we will make no distinction between the selectivity of AMPs towards bacterial cells and the selectivity of AMPs towards anionic membranes.

Specificity towards bacterial membranes in favor of mammalian membranes is a highly desirable functional characteristic of AMPs. However, both natural and laboratory synthesized AMPs have a broad range of specificities. The selectivity of AMPs to bacterial cells in preference to mammalian cells has been attributed to differences in the lipid composition of the plasma membranes of human erythrocytes (representative of most human cells) and those of bacteria. One hypothesis is that the presence of cholesterol, which makes mammalian membranes mechanically resilient to AMPs [44]. Presence of cholesterol increases the compressibility modulus and the bilayer bending modulus of PC bilayers, making them resistant to AMP-induced perturbation. Secondly, bacterial inner membranes consist of a significant proportion of anionic lipids (PG and PS lipids) while the mammalian inner bilayer membrane consists of neutral zwitterionic PC phospholipids [45].) Several measurements have suggested that cationic AMPs have lower affinity to zwitterionic lipids than anionic lipids. The preferential electrostatic binding of AMPs to anionic interfaces thus partially explains the selectivity of AMPs towards anionic membranes [46]. However, not all cationic AMPs are selective towards anionic interfaces. Thus, depending on their amino acid composition, different AMPs target membranes of different lipid composition.

The chemical nature of membranes can modulate the structure, and consequently the functional specificity of AMPs. We will show that membranes of various lipid compositions mould peptide properties in different ways, which in turn influences the extent to which a membrane of a particular composition is perturbed by a peptide.

It is well-known that most helical AMPs are unstructured in aqueous media, and acquire helical form after membrane binding. The extent of helical content induced in AMPs in presence of the membrane interface influences the extent of binding to and perturbation of the membrane [47]. Only a few studies have compared the extent of AMP helix induction in anionic and zwitterionic membranes; these are summarized in Khandelia et al. [48]. In the following, we will show using simulations that membranes alter peptide structure which in turn has a significant impact on the extent of binding of peptides to membranes and subsequent membrane permeabilization.

Ovispirin-1 (OVIS) is an 18-amino acid (KNLRR IIRKI IHIIK KYG) helical AMP which is highly antimicrobial but also cytotoxic [47]. Novispirin G10 (KNLRR IIRK**G** IHIIK KYG) and Novispirin T7 (KNLRR I**T**RKI IHIIK KYG) are single residue mutants of OVIS which retain antibacterial activity, but are less cytotoxic [12]. Thus, OVIS binds both zwitterionic and anionic interfaces, while the mutants preferentially bind anionic interfaces. Three-dimensional structures evaluated in trifluoro-ethanol (TFE) showed that the point mutations reduced the helical content of G10 and T7 [12]. The structural and functional properties of the three peptides, and their similar charge (+7) make them suitable to carry out investigations of the interplay between membrane-influenced peptide structure and peptide–membrane interactions. MD simulations of the three peptides were implemented in zwitterionic dodecylphosphocholine (DPC) and anionic sodiumdodecylsulfate (SDS) micelles [49].

Pair distribution functions and binding enthalpy calculations indicated that the strength of interaction of polar amino acid side chains with DPC and SDS headgroups was comparable [49]. The side chains of the cationic and polar amino acids on the peptides formed H-bonds with the SDS or DPC headgroups [49].

OVIS, the parent peptide, remained helical in DPC, while both T7 and G10 became less helical compared to their structure in TFE in DPC (Table 1). In SDS, all three peptides essentially retained their secondary structural content. (Later, NMR and CD spectroscopy revealed that G10 was indeed more helical in presence of SDS micelles, compared to DPC

Table 1
Fraction of helical residues in TFE, SDS and DPC correlates with the distance between the center of mass of the micelle and the peptide

Peptide	Fraction of helical residues			$r_{pep}-r_{micelle}$ (Å)	
	TFE	SDS	DPC	DPC	SDS
OVIS	0.9375	0.8750	1.00	16.2	16.0
G10	0.6875	0.6250	0.500	19.1	15.9
T7	0.6875	0.6875	0.5625	20.3	16.2

For TFE, the fraction of helical residues was obtained from the NMR structures.

micelles, thus confirming the simulation prediction [50]). The extent of binding to zwitterionic DPC and anionic SDS was measured by the depth of insertion of the peptides into the interface. In DPC, OVIS inserted deepest into the interface, while in SDS, all three peptide remained equidistant from the micellar center of mass. The comparative depth of insertion of the three peptides in SDS correlates with their comparable affinity to bacterial anionic membranes. The greater depth of insertion of OVIS in DPC correlates with its higher affinity towards mammalian zwitterionic membranes (OVIS is more cytotoxic compared to G10 and T7). The secondary structure content of peptides and the extent of binding to DPC are thus correlated. The stabilization of the helical structure of OVIS in the presence of DPC preserved peptide amphiphilicity, such that hydrophobic amino acid residues could bind cooperatively to the interface. The loss of helicity of G10 and T7, led to lower amphiphilicity resulting in a lower depth of insertion into the micelle. The point mutations in G10 and T7 thus induced changes in peptide secondary structure that prevent the cooperative isolation of hydrophobic residues into hydrophobic core of the micelle.

Simulations cited here suggest that lipids do not play only a secondary role in determining AMP selectivity. The composition of a target membrane (percentage of acidic phospholipids present) modulates the extent of helical content induced in AMPs. The level of secondary structure induced in different peptides ultimately determines their selectivity profile. Fig. 4 summarizes this complex relationship between membrane-induced peptide conformation and peptide-induced membrane perturbation. In the conventional view of AMP-induced membrane perturbation, zwitterionic and anionic membranes induce similar secondary structure in peptides. Simulations propose a modified perspective, where the membrane has as much influence on peptide conformation as the peptide has on membrane integrity.

4. Peptides with hydrophobic anchors

A common strategy used by peripheral and integral proteins to associate with membranes and engage in signal transduction is to exploit various hydrophobic anchors to facilitate binding to the membrane and to localize in particular regions of the membrane such as lipid domains, rafts, and caveolae [51–53].

Typical hydrophobic anchors are acyl (myristoyl, palmitoyl) or prenyl (linear poly-isoprene) chains. The affinity of the protein for various membrane domains can then conveniently be altered by enzymatic cleavage of the anchor. Examples of peripheral proteins that anchor themselves to membranes include Ras proteins, Lamin B and coat proteins, protein kinase C substrate and Src proteins, HIV-1, and Nef. The anchoring strategy can be exploited in biomedical and pharmaceutical context to enhance the efficacy of certain peptide drugs [54,55] and hormones such as insulin [56]. The anchoring of a small water-soluble peptide to a lipid membrane is of particular interest. The anchor forces the peptide to localize at the membrane–water interface and as a consequence both the peptide structure and the lipid-bilayer structure and dynamics are altered. Structural changes in the peptide can alter its possible function and e.g. binding to receptors. Structural and dynamical changes in the lipid bilayer may reflect changes in domain organization and lipid acyl-chain ordering.

In a combined experimental and MD simulation study, these effects were studied for a specific cationic decapeptide, anchor-HWAHPGGHHA-amide, with N-terminal acyl anchors of different length, specifically C2, C8, and C14 [43,54,57]. The system is illustrated in Fig. 5. Due to the proline residue in the middle of the peptide it forms a hairpin structure. The tryptophan residue assures that the peptide becomes localized at the water–membrane interface. It was found as expected that the

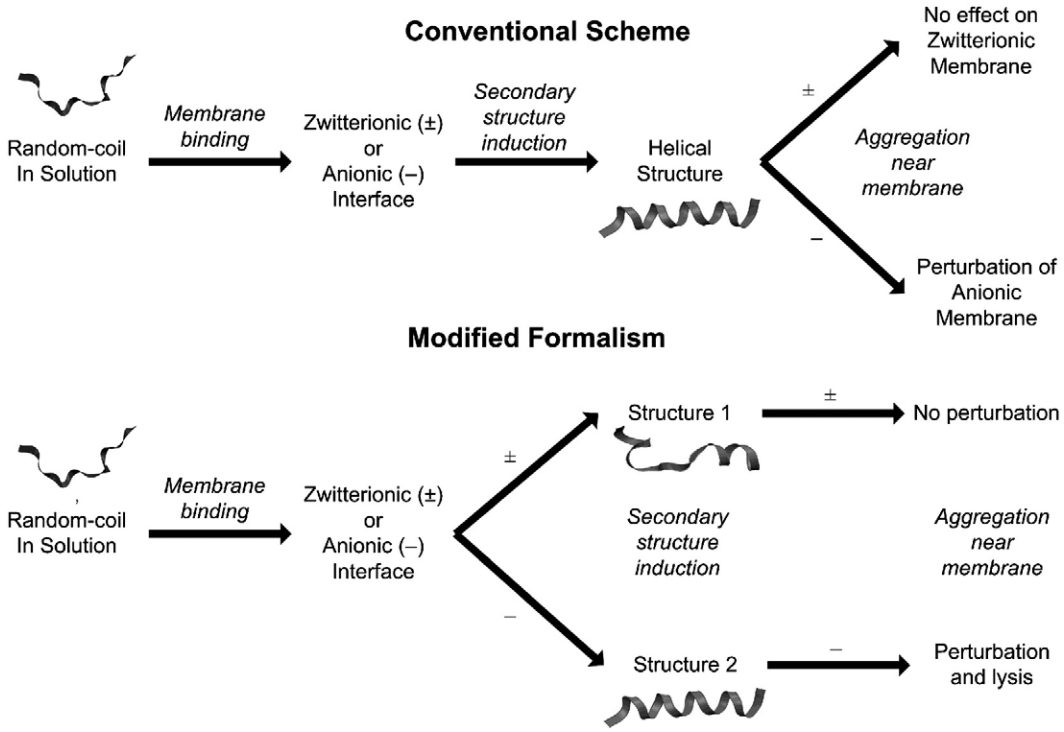

Fig. 4. Top: The conventional view of AMP-induced membrane perturbation, where zwitterionic and anionic membranes induce similar secondary structure in peptides. Bottom: Simulations propose a modified perspective, where the membrane has as much influence on peptide conformation, as the peptide has on membrane integrity.

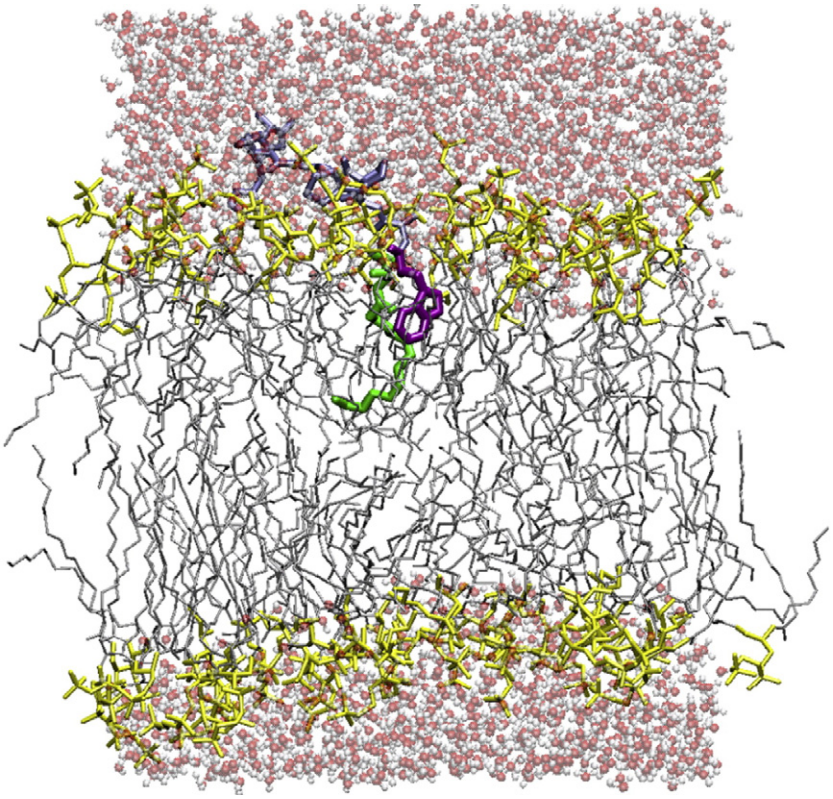

Fig. 5. Snapshot from a MD simulation of a myristoylated decapeptide, C14-HWAHPGGHHA-amide, anchored into a DPPC lipid bilayer. The anchor is shown in green, the interfacial tryptophan residue in purple, and the remaining peptide in blue. During the course of the simulation, the anchor performs extensive excursions in the bilayer. Adapted from Ref. [57].

longer the acyl chain, the stronger the binding of the peptide to the bilayer and the larger influence on the lipid phase behavior and lateral organization of the bilayer.

In particular, the peptide with the longer anchor, C14, is found from the simulations to have a significant effect on the bilayer. Despite the long anchor, the peptide is highly mobile at the membrane surface and its motion is integrated in the collective dynamical modes of the bilayer. The peptide maintains high conformational flexibility and exhibits a disordering effect on the bilayer and a concomitant thinning and lateral expansion of the membrane. The disordering effect is quantified in Fig. 6 which shows the acyl-chain order parameter along the chain together with the order parameter of the myristoyl anchor chain. The disordering

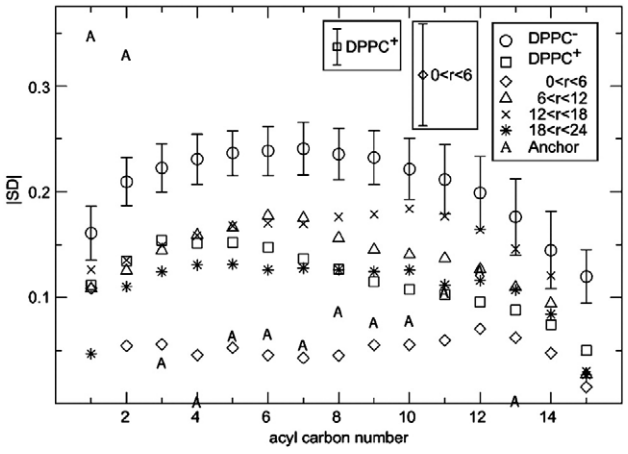

Fig. 6. Acyl-chain order parameter profile obtained from MD simulation of a myristoylated decapeptide, C14-HWAHPGGHHA-amide, anchored into a fluid DPPC lipid bilayer. Data are shown for a bilayer without ($DPPC^-$) and with ($DPPC^+$) a peptide incorporated. The different data sets are explained in the inset and show the order in different distances r (measured in Å) from the peptide. Adapted from Ref. [43].

effect is most pronounced in the region from carbon number 2 to 8. Inspection of the dynamics of the peptide indicates that this effect is caused by a curling of the anchor towards the interface and a substantial wobbling of the peptide. Therefore, both the static and the dynamic order of the lipid membrane are affected by the acylated peptide.

4.1. Prenyl anchors vs. acyl anchors

The effect of prenyl chains and acyl chains on the membrane properties can be drastically different, and this is one reason why these two groups of membrane anchors have received considerable attention in recent years. It appears that prenyl chains provide a weaker binding to phospholipid vesicles than saturated acyl chains [58,59]. In a DMPC membrane in pure water small, neutral compounds with lipidic anchors are insoluble in water [60–62] and fully partition into the membrane. MAS-NOESY NMR studies further confirm that both acylated and prenylated peptides are structurally organized in the membrane as expected with the lipidic chains aligned with the phospholipid acyl chains and the hydrophilic moieties in the interfacial region [61,63]. But the two types of lipid anchors affect the membrane properties very differently. This is for example, reflected in the way anchors affect the *main transition*. While the small components with a myristoyl chain like monomyristoylglycerol [64], increase the main transition significantly and stabilizes the low-temperature s_o phase, the small membrane compound with a farnesyl chain (linear poly-isoprene 3-mer), like farnesol, has the opposite effect and is stabilizing the fluid l_d phase [62]. These different affinities to the ordered and disordered lipid environments found in thermodynamic studies also show up in fluorescence microscopy [65] and partition studies [59] and have therefore become candidates for the main sorting mechanisms between l_d and s_o domains ("rafts") in the membranes [66]. Studies of the effect of farnesyl anchors on the mechanical properties of DMPC membranes have further revealed that the bending rigidity is hardly changed [61]. Even 25 mole%

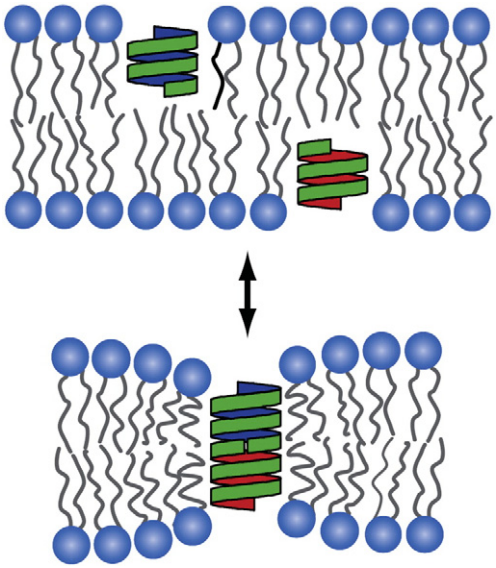

Fig. 7. Illustration of the process of dimer formation of gramicidin A monomers in a lipid bilayer leading to a conducting membrane-spanning channel. In the case shown, the dimer is shorter than the bilayer thickness resulting in a local deformation of the bilayer near the channel. Courtesy of Dr. Olaf Sparre Andersen.

farnesol has very little impact on the bending rigidity. This is consistent with 2H NMR results which shows that the lipid chain order is hardly affected up to 20 mole% farnesol in the membrane [62].

Numerous membrane proteins contain both prenyl and acyl chains. The biophysical studies thus support that enzymatic attachment and removal of lipidic chains have the capacity to direct and redirect proteins to specific membrane environments in the cell and within a membrane. Further, the mixed chain peptides may act as surfactants in the plane of the membrane, stabilizing micro-domains or forming a two-dimensional micro-phase separation (or micro-emulsion), as fluorescence microscopy studies indicate [67].

5. Small channel-forming peptides: the case of gramicidin A (gA)

One of the most well-studied poly-peptides is the antibiotic gramicidin A (gA) which in its natural form secreted by *Bacillus brevis* is a hydrophobic pentadecapeptide consisting of an alternating sequence of D- and L-amino acids forming a β-bonded helix in a hydrophobic environment. gA was the first antibiotic used clinically as early as in 1939. This small protein is at the same time the peptide whose effects on lipid-bilayer membranes have been described in the greatest quantitative detail. Similarly, the effect of the lipids on the functional properties of gA is the type lipid–protein interaction which most clearly has demonstrated how lipids impact on protein function [68,69].

gA incorporates easily into lipid membranes where it can form a dimer channel bound by six hydrogen bonds between the opposed N-termini. The channel spans the bilayer and selectively conducts a current of small cations at a rate of 10^7 ions per second. Hence its function is to form a well-defined conducting pore as shown in Fig. 7. The system can be viewed as a two-state protein with a closed state (monomers) and an open state (dimer). The position of the monomer–dimer equilibrium can be measured very accurately by electrophysiological techniques down to the single-channel level since it appears that the conductance of the intact dimer is not affected by stresses imposed by the lipid bilayer [70,71]. In addition the lifetime statistics of the dimer can be determined. The interaction between gA and lipid bilayers have most often been analyzed theoretically in terms of the hydrophobic mismatch that may pertain between the hydrophobic length of the dimer and the hydrophobic thickness of the lipid bilayer (see Ref. [69] and references therein). It turns out that the analysis allows for a quantitative description that treats gA as a mechanical transducer that picks up and reports on the elastic distortions in the bilayer in terms of thickness changes and curvature stress induced in the lipid annulus around the channel. Hence even the mechanics of a biological membrane can be probed by an appropriate assay involving gA and electrophysiological measurements.

gA in lipid bilayers lends itself to be studied by the same type of molecular modeling and MD techniques that have been used to study integral membrane proteins in membranes [72–74]. The simulations have mostly addressed molecular properties of the channel [75–79] and hence only small membrane patches with few lipid molecules have been considered. Only few studies have focused on the modulation of the lipid-bilayer matrix itself. Since the time scale of ion flux through the channel is of the order of 10–100 ns it is at present difficult to quantitatively relate structure to function via an atomic-scale MD simulation [78]. In the simulations, the lipid patches used usually correspond to from one to three shells of lipids around the channel. A key result from these studies is that the formation of a single file of water molecules through the channel is decisive for ion conduction [75]. gA has generally an effect on the lipid bilayer that depends on the degree of hydrophobic mismatch. It was found in early studies, that in thick bilayers gA even destabilizes the bilayer structure and induces hexagonal phases [80]. It has remained controversial, however, to which extent the lipids adapt to the hydrophobic thickness of the channel. Based on the general theory of hydrophobic matching [69,81] one would expect that e.g. in thick membranes, cf.

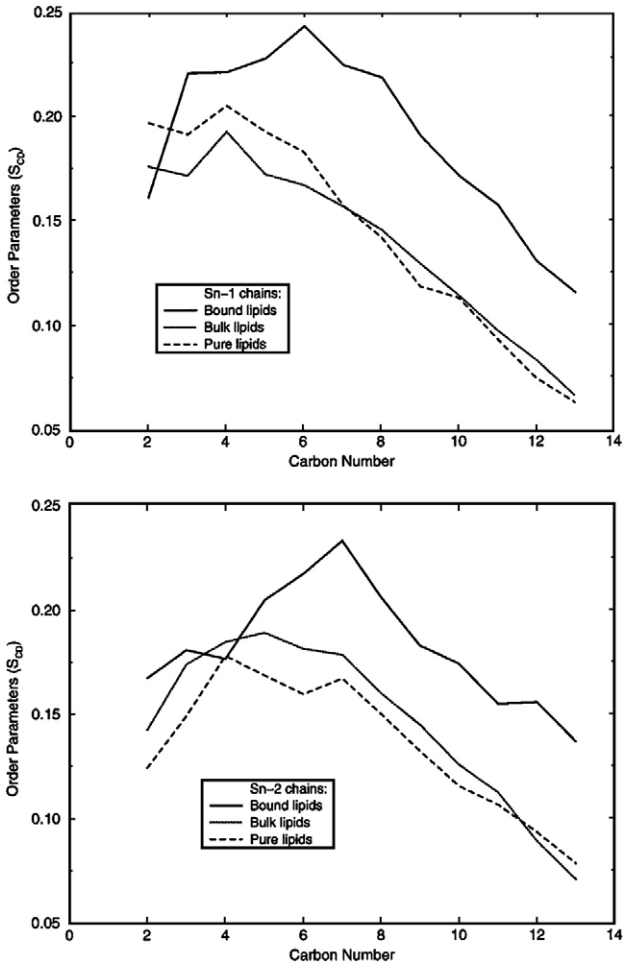

Fig. 8. Acyl-chain order parameter profiles obtained from MD simulation of a gramicidin A dimer channel incorporated in a fluid DMPC bilayer. Data are shown for the *sn*-1 (top) and *sn*-2 (bottom) chains separately. A bound lipid is a lipid that either directly or through a water molecule is hydrogen-bonded to the channel. A comparison is made with a pure bilayer (dashed lines). The different data sets are explained in the insets. Adapted from Ref. [82].

Fig. 8, the lipid molecules near the channel would become disordered in order to become shorter. However, the boundary condition imposed by the channel may be more complex on the molecular scale than is anticipated by a simple continuum elasticity theory. Furthermore, details of the lipid molecular structure may also play a role.

MD simulations on DMPC bilayers which correspond to the situation depicted in Fig. 8 showed that contrary to expectations the acyl-chain order parameters of the lipids adjacent to the gA channel were higher than in the bulk [82,83]. The ordering of more distant lipids seems not to be affected. The simulation results are in accordance with the interpretation [77] of early NMR work [84].

More recent MD simulation studies of gA in GMO and DiPhPC bilayers point to the opposite effect [85]. In GMO bilayers, the acyl chains were found to disorder near the channel, consistent with experimental studies on the same system [86]. The DiPhPC bilayers seem not to be affected, probably because of the branched structure of the chains. The apparent disagreement between the simulation results for DMPC and GMO bilayers may be found in the difference in the interfacial structure in phospholipid and glycerol bilayers. Moreover there are subtleties related to the hydrogen-bonding between the interfacial tryptophan residues of gA and the glycerol backbone and/or water molecules. In fact, EPR work suggests that the tryptophan residues may immobilize or restrict the motion of four lipid molecules [87]. This restriction could possibly present a boundary condition that induces acyl-chain order. Finally, there may be time-scale considerations to be made. For large systems, MD simulations are at best extended to tens to hundreds of nanoseconds with reasonable computer resources, whereas the time scales of NMR studies are in the μs-regime. It appears that more work is needed to assess the details of the effects of gA on the lipid bilayer near and away from the channel. Simulation results on larger trans-membrane proteins are more clear-cut on this point, possibly because of the larger circumference of these proteins. For example, MD simulation on the acyl-chain order in lipids around *Escherichia coli* aquaporins AqpZ and GlpF in fluid POPE and POPC bilayers [73,88] also shows an increase in the acyl-chain order parameter. Still the bilayer thickness, which readily can be calculated, decreases towards the protein and meets effectively the hydrophobic matching condition. The resolution to this puzzle was shown to be a tilting of the lipid chains next to the protein, again reflecting a very special molecular boundary condition.

6. Concluding remarks

One of the obvious limitations of most MD simulations cited in this review is the small size of the membrane patches and the limited time scales that can be sampled with current computer resources. Longer time and length scales have now become accessible with the development of accurate coarse-grained models for proteins and lipids which allow longer time steps, and have reduced degrees of freedom. The maturation of coarse-grained simulation methods will allow investigation of much larger systems (more peptides, larger membrane patches) for longer times. Such more extensive simulations will be especially useful for investigating the mechanism of action of AMPs. However, if protein secondary structures are invariant in the coarse-grained simulations, it is possible that some free energy barriers will remain unconquered, and a global energy-minimized pore conformation is not attained. Nevertheless, in the next few years, it will be reasonable to expect coarse-grained simulations to provide fascinating new insights into the mechanism of interaction and binding of AMP-mediated pore-formation in membranes. Moreover, it will also be possible to investigate larger systems even with fully atomistic simulations with the advent of faster computers and better networking hardware for parallel computing.

It is expected, as parallel simulation algorithms improve and the computer capacity becomes extended, that the study of the interaction of peptides and small proteins with membranes will be brought to a stage where they come to play a seminal role in the rational development of peptides as drugs. Such simulations are also likely to gain importance for guiding the development of drug-delivery systems based on particular lipid systems, such as micelles and liposomes.

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