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Review

A dynamic view of peptides and proteins in membranes

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Abstract. Biological membranes are highly dynamic supramolecular arrangements of lipids and proteins, which fulfill key cellular functions. Relatively few high-resolution membrane protein structures are known to date, although during recent years the structural databases have expanded at an accelerated pace. In some instances the structures of reaction intermediates provide a stroboscopic view on the conformational changes involved in protein function. Other biophysical approaches add dynamic aspects and allow one to investigate the interactions with the lipid bilayers. Membrane-active peptides fulfill many important functions in nature as they act as antimicrobials, channels, transporters or hormones, and their studies have much increased our understanding of polypeptide-membrane interactions. Interestingly several proteins have been identified that interact with the membrane as loose arrays of domains. Such conformations easily escape classical high-resolution structural analysis and the lessons learned from peptides may therefore be instructive for our understanding of the functioning of such membrane proteins.

Keywords. Protein-lipid interactions, GPCR, channel, pore, Bcl-2, colicin, alamethicin, melittin.

The classical picture

Biological membranes are complex supramolecular assemblies that have attracted considerable attention as many processes of cellular signaling, metabolism and transport occur at the surface and within these delicate structures. although they are efficient barriers for hydrophilic solutes and pathogens, they allow the highly regulated passage of substances and information. Our present-day view of the membrane structure is largely based on the fluid mosaic model published by Singer and Nicholson in 1972 [1], where the lipid bilayer forms a hydrophobic matrix about 5 nm in thickness in which a variety of proteins with specific functions are embedded in a mosaic-type fashion. Whereas movements are much restricted within the direction of the membrane normal, lateral diffusion within the plane occurs in a manner comparable to a viscous fluid. The dimensions and structure of lipid bilayers have been confirmed by neutron diffraction and solid-state NMR techniques [2–4], and more recent investigations, including magic-angle spinning (MAS) solid-state NMR and molecular modeling, add further interesting detail and indicate considerable conformational flexibility of the lipid molecules within the bilayer [5].

In the past, the structural investigation of membrane proteins has been hampered due to their amphipathic or hydrophobic character, problems of solubility in aqueous buffers, and their limited stability in the absence of lipid bilayers. All these factors make it difficult to obtain the quantitative amounts of proteins needed for structural investigations. In addition, among those systems that can be made available many are too large for a detailed solution NMR spectroscopic analysis and/or they are difficult to

crystallize in their native conformation, a requirement for high-resolution X-ray diffraction techniques. Therefore, the number of membrane protein structures that is available falls much behind those of soluble proteins ([6, 7] and/or http://blanco.biomol.uci.edu/Membrane_Proteins_xtal.html), although a number of structural models at lower resolution have also been obtained by microscopic techniques [8–12]. Notably this is not related to the lack of abundance or of biomedical importance, as the statistical analysis of the genome of several species indicates that about 30% of all expressed reading frames encode for membrane proteins [13, 14]. Furthermore, within the available membrane protein structures, those sequences that already naturally show a tendency to form crystalline arrays are probably overrepresented. In particular, very few crystal structures of G protein-coupled receptors have so far been solved [15, 16], although this family of proteins is of major importance in pharmaceutical and medical research. Worldwide many research groups in academia and industry are interested in developing better and reliable approaches for the over-expression and purification of members of this family of proteins [17–20].

The existing membrane protein structural database is characterized by proteins whose sequences form helical bundles or beta barrels within the lipid bilayer. Such tertiary structures are favorable in hydrophobic environments as they avoid the energetically unfavorable exposure of unsaturated hydrogen-bonding partners to the membrane environment [4]. The statistical analysis of transmembrane helical domains either from structural or biochemical topology data all indicate a strongly hydrophobic character of the amino acid side chains with a preference for aromatic amino acids at an interfacial belt [21]. However, some polar residues are also found in the bilayer interior or within the membrane-inserted protein domains as hydrophobic contributions can compensate for unfavorable polar interactions. Indeed hydrophobic model sequences exhibit transmembrane alignments despite the presence of histidine or lysine residues in central parts of the helix [21–23]. Furthermore, the salt bridges and hydrogen bonding interactions of the few polar residues found within transmembrane helical domains are often involved in the control of helix association and oligomerization [24–26].

A view of proteins at work and in their lipid environment

It has been possible to trap the protein structures of reaction intermediates of bacteriorhodopsin and thereby to obtain a stroboscopic view of the conformational changes during its photocycle [27]. In a related manner, several structures of the skeletal muscle sarcoplasmic reticulum Ca-ATPase [28, 29], and, by homology, of the *Arabidopsis thaliana* autoinhibited H^+-ATPase2 [30], reveal the conformational changes of these membrane proteins during ion passage and ATP hydrolysis. Furthermore, the changes in shape of the huge nuclear pore complex have been followed by cryo-electron microscopy [11]. Without the knowledge of such reaction intermediates or the characterization of the conformational assembly, which is a better description of the protein structure, it often remains difficult to trace the dynamical changes that are associated with protein function [16, 31]. Alternative biophysical approaches that have been used to investigate the structure, dynamics and conformation of membrane proteins include fluorescence spectroscopy, through which distances and membrane penetration can be assessed [32, 33], circular dichroism and infra red spectroscopies, which allows monitoring of protein conformation, picosecond dynamics and topologies [34–38], electron paramagnetic resonance and solid-state NMR spectroscopy for assessing polypeptide dynamics, topology and interactions [39–42], and isothermal titration calorimetry, which has been used to quantitatively study the thermodynamics of polypeptide membrane association [43].

Whereas structural biology techniques reveal amazing details on protein structures, considerably less is known of the surrounding membranes. Therefore, the positioning of the proteins relative to the lipids is often based on assumptions and guesswork. To visualize the protein structures in their membrane environment it has therefore been necessary to trace the outlines of the bilayer structure in an intuitive manner. Only in a few cases was it possible to obtain deeper insight into the protein-lipid interactions by, for example, the observation of the electron density originating from lipids associated with the proteins [16, 44, 45]. Furthermore, intermolecular NMR cross peaks (NOEs) and the effect of paramagnetic relaxation reagents have been used to outline the interaction surfaces between OmpX and the micellar detergent 1,2-hexanoyl-*sn*-glycero-3-phosphocholine (DHPC) [46, 47]. Using solid-state NMR techniques specific lipid-protein interactions involving polyunsaturated fatty acyl chains and phosphatidylethanolamine head groups have been detected that are important during rhodopsin activation [48].

Other biophysical studies have shown that the membrane exhibits highly dynamic features and its dimensions and shape can vary [49]. Not only do the lipid head groups and fatty acyl chains adopt a range of

conformations and alignments within the membrane [5, 50, 51], the membrane as a whole bends and expands, and the possibility exists that non-bilayer structures form [52, 53]. When these transitions occur locally and transiently they are difficult to visualize in a direct manner, but functional assays such as pore formation or membrane fusion provide indications that they exist [54–56]. However, at higher polypeptide/lipid ratios local or global transitions in the membrane macroscopic phase properties have been monitored by structural techniques [52, 57]. Although it is widely accepted that a dynamic membrane involving such changes of the macroscopic phase properties are essential to explain the functioning, *e.g.*, of antimicrobial or fusion peptides, this is much less the case when larger proteins are considered.

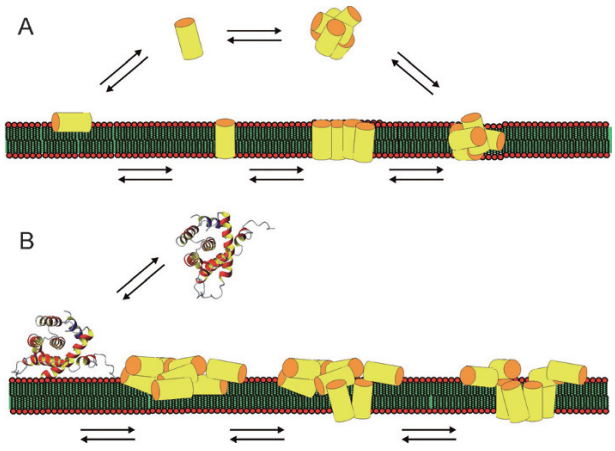

Figure 1. (*A*) A variety of equilibrium configurations of peptides in their soluble and membrane-associated states are shown. Whereas the peptides in solution occur in monomeric, oligomeric or aggregated forms, they adopt in-plane and transmembrane alignments and various aggregate shapes in the membrane. Note that not all peptides adopt all of the configurations shown and that there might be other possible arrangements. Furthermore, in the solution state, depending on their concentration, the peptides often adopt random coil conformations rather than the helical structures schematically shown. (*B*) The pore-forming domains of several colicins as well as Bcl-2 proteins adopt globular folds in solution and associate with the membranes in response to changes in pH or other signals. Whereas the overall global fold of some proteins such as Bcl-x_L seems to largely remain intact at physiological pH when associating with membranes, at acidic conditions the colicin E1 channel domain has been shown to adopt a more open conformation with most helices lying parallel on the membrane surface (a loosely connected helical array). To represent the soluble and superficially associated configurations of the Bcl-2 and the colicin families a ribbon drawing is shown (1BXL). Furthermore, the membrane-inserted states, as suggested by the colicin pen-knife and umbrella models as well as a configuration involving membrane insertion of other helices, are schematically represented from left to right. Although the membrane is shown as a bilayer slab, it should be kept in mind that other macroscopic phases can occur transiently and locally or globally in the presence of polypeptides (cf. text for details and Fig. 2).

Is the classical view on membrane proteins complete?

By emphasizing those structures that are naturally or artificially abundant, that behave in a favorable manner during the biochemical purification and that have a good tendency to crystallize, the known structural database is probably biased and misses out on tertiary membrane structures that are not folded in a compact manner or that are toxic to cells when overexpressed in large amounts. For example, the C-terminal domains of several colicins have been shown to insert into bilayers and to form voltage-gated channels in black lipid membranes, a process involving significant structural transitions [58–60]. In solution the colicin pore-forming domains share structural similarities not only with each other but also with other bacterial toxins, such as proteins secreted in diphtheria, tetanus and botulinum, the *Bacillus thuringensis* δ-toxin, or with the Bcl-2 family of proteins, the latter being key regulators of apoptosis [61, 62]. The three-dimensional structures of a number of members of the Bcl-2 family (Fig. 1B) as well as of the channel-forming bacterial toxins have been determined by X-ray diffraction and solution state NMR spectroscopy and represent the proteins in their soluble state, *i.e.*, before insertion into the membrane. Their common globular fold is characterized by eight to ten α-helices arranged in a three-layered structure where the central layer is composed of a hydrophobic helical hairpin, which is surrounded by a layer of amphipathic helices on each side of the resulting 'sandwich' [62].

During membrane insertion the colicin channel domains undergo pronounced conformational changes, adopt intermediate conformations, and finally insert into the membrane by refolding to better match the bilayer interfacial properties [63, 64]. Based on experimental evidence the resulting membrane structures of the channel-forming colicins have been described by two models (Fig. 1B). The first is the umbrella model, in which the two hydrophobic helices are arranged in a transmembrane orientation and the amphipathic helices intercalate into the interface [61], a model later refined to be a dynamic array of loosely connected helices [63]. The second is the penknife model, in which the helices all orient approximately parallel to the surface and form tightly packed structures [59]. Experimental evidence exists in support of the umbrella model for colicin E1 [60] or colicin Ia [65], and in support of the penknife model for the colicin A channel [59]. More recently experimental data have been presented indicating that various membrane conformations of the colicin E1 and Ia channel domains can co-exist and are related with each other [66, 67]. In a similar manner,

structural transitions have also been observed for the diphtheria toxin T domain [68]. Such membrane structural arrangements of the protein helices agree with a model in which the lipids are part of the channel lining [60] analogous to the propositions made for amphipathic antimicrobial peptides [69].

The solution structures of several members of the Bcl-2 family, regardless of their pro- or anti-apoptotic activities, exhibit close relationships to those obtained from the channel-forming bacterial toxins discussed above [70]. Indeed channel activities have been measured for the anti-apoptotic Bcl-x_L [71] and Bcl-2 proteins [72]. The openings resemble those of colicins in that they are discrete, cation selective and pH dependent. In contrast, the permeability changes induced in planar lipid bilayers by the pro-apoptotic Bax are arbitrary and continuously variable [73]. Bax also exhibits lytic activities against neuronal or red blood cells as well as releasing fluorescent dyes from liposomes [74].

The Bcl-2 family of proteins is an important factor during the regulation of controlled cell death and, by being involved in a variety of disorders including cancer, autoimmunity and neurodegeneration, are of great biomedical interest [75]. The proteins form a complex interaction network where the activity is regulated by protein-protein and protein-membrane interactions concomitant with the re-localization of the proteins from the cytoplasm to organellar membranes [75]. It is generally believed that Bax, a pro-apoptotic member of the family, is targeted to the mitochondrial outer membrane where it forms pores and that these are important steps during a cascade of regulatory interactions and events. However, despite their primordial bio-medical importance only a few studies have been conducted to reveal their conformations when associated with membranes.

When the structure and interactions of the anti-apoptotic Bcl-x_L were investigated in the presence of membranes by solid-state NMR spectroscopy the data agreed with a type I membrane protein where the global fold encompassing helices 1–8 remains intact (Fig. 1B) and where the most C-terminal helix anchors the protein in the membrane [40]. Membrane insertion is thus correlated with conformational changes which upon interaction of the C-terminal helix with the membrane liberates a hydrophobic binding pocket for interactions with other members of the Bcl-2 family. Interestingly, when investigated independently, the hydrophobic C-terminal domain can adopt in-planar as well as transmembrane alignments depending on the detailed environmental conditions, suggesting that membrane anchoring of Bcl-x_L is reversible. It is tempting to speculate that the pore- and membranelytic structures of Bax resemble more closely the helical arrays of membrane-active colicins, but so far little structural information is available about this protein in its membrane-associated state.

Although variable alignments have also been observed when investigating membrane-associated peptides [40, 76], this concept is less common for larger membrane proteins. However, it remains possible that such loosely folded membrane structures occur more often but escape our attention as such conformational flexibility is difficult to visualize by crystallographic techniques. The investigation of the peptide-membrane interactions is therefore not only of interest to understand the functional mechanisms of antimicrobial action, transfection or fusion by these compounds, but also in the context of larger proteins. Some proteins might form helical arrays as has been suggested for colicins or related open conformations where individual secondary structure elements are loosely connected by flexible loop regions. Notably, electron densities are often missing in high-resolution membrane protein structures, and many of these 'invisible' domains are probably flexible and dynamic and some of them may potentially interact with the membrane.

The study of peptides provides a more dynamic view

Membrane-active peptides have been studied extensively by biophysical approaches as they carry out important functions such as signaling or the protection of the host organism against antimicrobial attack. Furthermore, they are considered to provide valuable insights into the interactions that govern the structure and function also of membrane proteins. Importantly, the picture that has emerged from such biophysical investigations is a rather dynamic one with multiple equilibria governing the interactions and conformations of these polypeptides with the membrane itself but also among each other (Fig. 1A).

For example, when the dodecameric hydrophobic alamethicin or related peptides are added to planar lipid bilayers, voltage-dependent conductance changes similar to those seen in the presence of large voltage- or ligand-gated channel proteins are observed (reviewed *e.g.* in [77]). The open alamethicin pore has been suggested to consist of 'transmembrane helical bundles', which are composed of helices grouped around a water-filled pore [78]. Similar arrangements have in the meantime been observed for a variety of membrane proteins including potassium channels, the acetylcholine receptor, the influenza proton channel or the phospholamban pentamer [9, 45, 79–82]. Helix-helix interactions are also key during the regulation of membrane protein activity

and transmembrane signaling of, for example, T cell receptors, integrins, tyrosine kinases and the MHC class II assembly. The detailed biophysical investigations of a glycophorin peptide and its dimerization motif GXXXG has much deepened our understanding of membrane helix-helix interactions [26, 83, 84]. Structural investigations indicate that alamethicin exhibits a preference for helical conformations with a flexible hinge region around proline-14 [85–87] and transmembrane alignments are observed by oriented solid-state NMR spectroscopy [88–90]. Notably, a variety of biophysical investigations indicate a high degree of conformational flexibility where the degree of helix formation is dependent on the physical state of the lipid, the lipid-peptide ratio, the presence of transmembrane potentials and other environmental parameters (reviewed *e.g.* in [91, 92]).

The pattern of successive conductance levels observed when alamethicin is added to planar lipid bilayers, each a few milliseconds in duration, suggests that the smallest conducting structures consist of trimers [93], tetramers [94] or pentamers [95]. The activation energies for the initial formation and decay of the lowest conductance state are 50 and 120 kJ/mole, respectively. Once this first channel structure has been formed the addition and subtraction of additional units occurs in a very dynamic manner on a millisecond time scale. Starting from the lowest observed values the conductivity of the black lipid membranes increases fast and in well-defined steps of a few hundred pico-Siemens.

When added to preformed membranes, monomeric alamethicin exhibits rather low membrane partitioning constants (in the 10^3 M^{-1} range), but as several membrane-inserted states coexist the fraction of overall membrane-associated peptides is quite high. Using biophysical approaches it has indeed been observed that alamethicin and related peptaibols can adopt in-planar as well as transmembrane (TM) alignments, with the latter being favored at high peptide-to-lipid ratios [96].

It has, therefore, been suggested that the in-planar state of alamethicin is an intermediate during membrane association and channel gating [78]. Various models for the molecular mechanism of alamethicin pore-formation are based on interactions of its helix dipole with the TM electric field (reviewed in [91]), where voltage gating involves one or several of reorientations of the dipole, enhanced partitioning of alamethicin into the bilayer and membrane insertion of the N terminus [97].

By analogy, the voltage-dependent movements and the realignment of the strongly amphipathic 'paddle domains' of potassium channels have been suggested to be responsible for voltage gating of these proteins [42, 45]. In this model, movements across large parts of the membrane hydrophobic interior explain the gating currents of 12–14 elementary charges. It should be noted however, that with a high-resolution structure of the closed channel being unavailable at this time, experimental evidence and simulations exists for alternative models where the voltage-sensing S4 domain remains within the proteinaceous environment [98, 99]. In one of these models, the gating charge is explained by much smaller conformational alterations. However, the structural changes are sufficient to reposition the charges of the voltage sensor into a very different isopotential environment by modulating its accessibility between inside and outside.

Membrane pore formation and/or discrete multi-level conductances have also been observed when amphipathic peptides, such as cecropins, magainins or melittin, are added to preformed bilayers (reviewed *e.g.* in [69]). Whereas the former two belong to the family of linear cationic peptide antimicrobials, the latter is a 26-residue peptide found in the toxin of the honey bee *Apis mellifera.* In contrast to alamethicin, the channels of magainins and cecropins are less well defined, and their properties suggest a rather dynamic and flexible supramolecular arrangement.

Amphipathic peptides are found in many species where they protect the host against a wide range of antimicrobial infections [100, 101]. There is now general agreement that these peptides directly interact with the phospholipid membranes rather than with specific receptors, although evidence is accumulating that some of them also have internal targets such as the membranes of organelles, DNA and enzymes [102, 103]. Nevertheless, the capacity of membrane-active peptides to disturb bilayer integrity, either by disruption or pore formation, has been suggested to be the underlying principle of both antimicrobial and toxic activities (reviewed *e.g.* [69, 104]) as well as entry to the cell interior [105].

In contrast to peptaibols, which adopt amphipathic but mostly uncharged structures in membranes, these peptides are strongly cationic. As a consequence, they are highly water soluble where they exhibit a tendency to adopt random coil conformations [69]. Helix formation has been identified as an important driving force favoring membrane association [106, 107]. A variety of methods, including oriented solid-state NMR spectroscopy indicate that amphipathic helices intercalate into the membrane with the helix axis oriented parallel to the membrane surface [69]. In this configuration the hydrophobic region is localized about 10 Å above the bilayer center in agreement with the amphipathic distribution of polar-charged and hydrophobic residues. The peptides thereby

reflect the interactions and topologies that have also been suggested for individual helices of the C-terminal domains of several colicins or of members of the Bcl-2 family of proteins [40, 63, 67, 108].

In addition to the equilibria that govern the polypeptide interactions within the membrane (in-plane ↔ transmembrane ↔ TM oligomers), monomeric and aggregated states also interchange within the water phase (Fig. 1A). For example, oligomers and/or self association have been observed for melittin, δ-lysine, designed peptide antibiotics as well as natural and synthetic acylated antimicrobial peptides (reviewed in [56, 109]). It is possible that these peptide oligomers have to dissociate before monomers can insert into the membrane or that the peptide 'micelles' interact directly with the lipid bilayer.

When added to preformed bilayers amphipathic peptides such as magainins and melittin insert into the membranes within 10–100 s depending on peptide concentrations. The kinetics of membrane insertion and pore-formation exhibits biphasic properties, which has lead to the suggestion that association and successive pore-formation are distinct steps [110, 111]. Notably, however, the penetration of quencher into the membrane interior already occurs during an initial phase when the peptide is aligned parallel to the membrane surface [112].

The equilibrated system of magainin 2 associated with zwitterionic phosphatidylcholines is characterized by a partitioning coefficient of about 2000 M^{-1} [113], which explains why in the dilute solutions used in optical spectroscopy association of this peptide to phosphatidylcholine (PC) membranes was virtually absent. To promote association, negatively charged phospholipids are required; these augment the apparent partitioning coefficient to 10^5–10^6 M^{-1} [114]. This is easily explained by the higher local concentration of positively charged peptide next to the anionic membrane surface. When the electrostatic interactions are considered separately the hydrophobic membrane partitioning reduces to 50–100 M^{-1} in the presence of 25 mol% 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol (POPG) [114]. In addition, it seems that association with anionic lipids is more superficial as their membrane permeabilizing activities are also reduced in the presence of negatively charged lipids when the number of peptides associated with the membrane is explicitly taken into account [115–117].

Another interesting class of membrane-active compounds are cell-penetrating peptides (CPP). The first ones identified were PAntp (*Drosophila* antennapedia transcription protein, later also named 'penetratin') and Tat (HIV-1 transcriptional activator protein) [118]. These sequences raised interest when it became apparent that they are capable of transporting large cargo molecules through membranes to the cytoplasm and the nucleus. Thereby they parallel the capacity of the diphtheria toxin T domain as well as the colicin A and colicin Ia channel domains to translocate large hydrophilic proteins into and across the membrane [119–121].

The CPPs are subdivided into three classes depending on their origin as well as their primary and secondary structure [122, 123]. A first class forms primary amphipathic structures, such as signal peptide nuclear localization sequence (SP-NLS) and transportan, where hydrophobic and hydrophilic cationic domains are separated along the amino acid sequence. The second class includes penetratin and designed sequences such as KLAL and RL16. These sequences have the potential to form amphipathic helices, *e.g.*, when interacting with membranes. The third class includes peptides predominantly carrying positively charged amino acid residues such as Tat or R_9. In this last category the accumulation of arginines is a striking feature and it has been suggested that the side chains of this residue associate with the sulfate and carboxylate groups of cell surface heparin sulfate proteoglycans [124, 125].

On the other hand, the amphipathic sequences exhibit many similarities with signal sequences and antimicrobial peptide antibiotics when their biophysical and functional properties are considered [118, 123, 126]. To date it is still a matter of debate how these transporter peptides (or proteins) enter the cell, but it seems that two independent mechanisms can be used, namely a direct membrane passage and endocytosis. Depending on the polypeptide properties and its cargo, one of them might be thermodynamically and kinetically favored and predominate over the other [127, 128]. Interestingly, molecular dynamics calculations show comparable pictures when the pore formation of Tat and magainin 2 are investigated [53, 129]. Notably the results correspond conceptually to a previously published model (see Fig. 3D in [69]). As it is difficult to draw clear lines between CPPs and linear amphipathic peptide antimicrobials, a new concept has emerged in which linear cationic peptides form a spectrum of compounds rather than distinct classes, and where the degree of cell penetrating – antimicrobial – lytic activities varies in a continuous manner [118].

Membrane polymorphism

The study of the peptide-lipid interactions of several amphipathic peptides has shown structural transitions upon membrane insertion as well as membrane topologies that are a function of peptide-to-lipid ratio and

other environmental factors (reviewed *e.g.* in [91]). Importantly, these investigations also reveal changes in membrane curvature and of lipid macroscopic phases due to the presence of the peptides. These effects have been characterized in considerable detail for melittin, which exhibits pronounced effects on the phase properties of dipalmitoylphosphatidylcholine (DPPC) at peptide concentrations as low as 0.1 mol%, indicating that the peptide exerts effects beyond its immediate neighborhood [91]. By inserting peptides into the headgroup region, the bilayer packing is disturbed within an estimated radius of approximately 50 Å [130, 131]. Deuterium and ^{13}C solid-state NMR measurements indicate a decrease in order parameter at the lipid bilayer interior for magainins and other amphipathic peptides [132, 133]. At high concentrations of magainin or melittin (>5 mol%), disk-shaped particles were found when mixed with 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), suggesting a detergent-like solubilization of the membrane, an effect that is enhanced below the gel- to fluid-phase transition [52, 91]. Although it seems improbable that such high peptide concentrations are present all over the cellular surface, it remains possible that under certain circumstances high peptide densities occur transiently and locally and have an effect on the membrane curvature.

Whereas melittin disrupts PC bilayers into small structures, the peptides exhibit bilayer-stabilizing effects when mixed with phosphatidylethanolamine, a lipid with known tendency for inverse hexagonal phases (H_{II}) [134]. A different behavior is again observed when melittin is inserted into charged phospholipid membranes such as cardiolipin, 1,2-dioleoyl- *sn*-glycero-3-phosphatidic acid (DOPA) or egg-phosphatidylglycerol where the peptide induces inverted macroscopic phases (H_{II} or cubic) [135, 136]. Electrostatic interactions are not only important during membrane association of cationic amphiphiles, they also have a pronounced effect on the lateral distribution of lipids within the bilayer. As in mixed model membranes, the cationic peptides preferentially interact with negatively charged membrane surfaces a segregation of the acidic phospholipids has been observed [137].

Interestingly, modulations of the membrane morphology have also recently been suggested in the proximity of the voltage-sensing domain of a chimeric Kv1.2/Kv2.1 potassium channel. The high-resolution structure of this membrane protein exhibits a localization of the amphipathic helical S3-S4 paddle domain, which is abundant in cationic residues, at the outside of the channel structure and in direct contact with the lipids [45]. With the paddle domain being fixed at two ends in the protein the lipids adopt a high degree of curvature and allows water to penetrate relatively deeply into the membrane.

Another interesting example is the transport of colicin domains (15–25 kDa) across the outer membrane of Gram-negative bacteria, which is mediated by their respective receptor proteins [138]. As the pores of these outer membrane proteins seem too restricted to allow for the passage of large proteins, it remains a puzzle how this is achieved? Recently, however, it has been demonstrated experimentally that colicin N binds to the periphery of OmpF, suggesting that the protein translocates along the protein-lipid interface. During such a process one would imagine that the membrane continuously remodels to allow for the passage of different protein domains.

Phase diagrams to describe the complex interactions

To take into account the full plasticity of phospholipid membranes when interacting with polypeptides, phase diagrams provide an adequate means to describe the wide range of structures, configurations and morphologies as a function of multiple parameters and conditions [69, 109]. These latter include the peptide-to-lipid ratio, the detailed membrane composition, temperature, hydration and buffer composition. Within such a phase diagram are regions that represent bilayers, slightly perturbed or stabilized in the presence of polypeptide, regions of lysis and membrane disintegration, but also regions where membrane openings form in a more regular manner and supramolecular arrangements represented by, for example, the wormhole [139] and the carpet models [140].

With the phase diagrams an important aspect of polypeptide-lipid interactions is re-introduced when compared to the static structural models that form the more classical view of membrane protein structure, namely the effects that polypeptides exert on membrane macroscopic phase properties. Such phase transitions can be transient and local, or they can affect the membrane as a whole. Furthermore, the lipid composition and the average thickness of the membranes might have an effect on the protein structure such as helix tilt angles and aggregation state [141–143].

These phase transitions and lipid–dependent interactions have been rationalized by the geometries of the molecules involved in membrane assembly. The nature of the molecules, such as their charge and hydrophobic volume, influences how strongly they interact, how deeply they insert and how much curvature strain they exert on the membrane [109]. These in turn are important parameters to control the

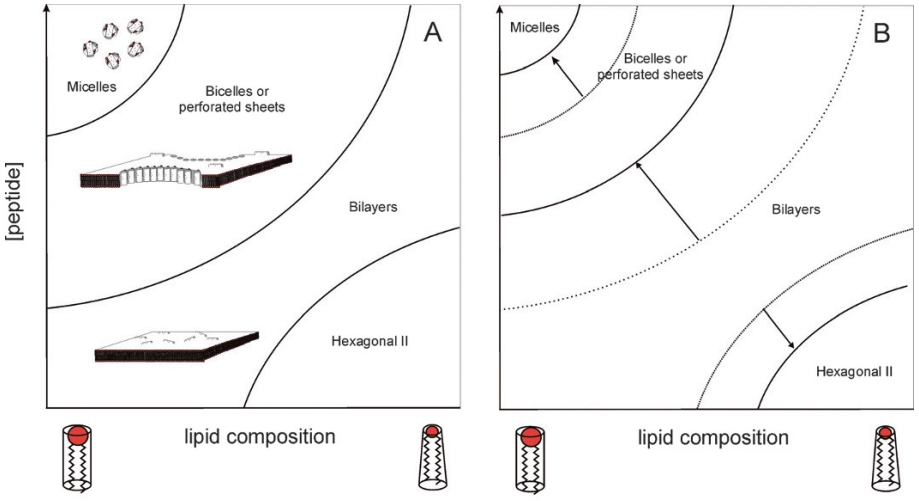

Figure 2. (*A*) A schematic phase diagram of a ternary mixture of two lipids and an amphipathic peptide. Whereas a cylindrical shaped lipid such as phosphatidylcholine forms stable bilayers, an inverted cone lipid such as phosphatidylethanolamine exhibits a tendency to form inverted hexagonal phases. In the presence of amphipathic peptides disrupted bilayer and micellar structures tend to form. The bilayer stabilizing properties of other lipids (such as cholesterol) is illustrated in (*B*); an effect that partially explains why eukaryotic membranes are more resistant to antimicrobial peptides when compared to bacteria [69].

wide variety of actions observed as well as the lipid polymorphism induced. When amphipathic helices intercalate into lipid bilayers they do not fill the volume at the level of the fatty acyl chains completely. Therefore, the peptide acts as a spacer at the level of the lipid headgroup and creates voids in the hydrophobic region of the membrane bilayer. As a consequence the hydrocarbon moieties compensate for such effects by chain bends, increased *trans-gauche* isomerization or chain interdigitation [109]. Therefore, not only the peptide molecular properties but also the size and shape of the lipid head groups and the membrane lipid composition have a pronounced influence on the membrane-peptide interactions (Fig. 2). 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 [109]. Thus, the molecular mechanism of membrane permeation and disruption depend on a number of parameters such as the nature of the peptides and membrane lipids, peptide concentration and environmental conditions.

Summary

A wealth of data indicates that the interactions between peptides and lipids are highly dynamic and that the membranes respond by adopting different morphologies. Whereas the membrane lipid composition has a pronounced effect on the amount of peptides associated, their penetration depth and their topology, the peptides in turn have the potential to modify the macroscopic phase properties of the membrane itself.

It is intriguing to note that proteins have been described where the partitioning between the membrane and the water phase constitutes an essential part of their biological regulatory mechanisms. In their membrane-associated form some of them resemble an array of loosely linked helices and the insights gained by the investigation of peptide-membrane interactions might be directly applicable to understand the function of these proteins. Furthermore, individual domains of otherwise tightly folded membrane proteins exhibit a rather dynamic character and therefore the structure, dynamics and topology of these domains might be modulated by the membrane lipids in a related manner.

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