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Interaction between amphiphilic peptides and phospholipid membranes

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Abstract

This brief review aims at providing some illustrative examples on the interaction between amphiphilic peptides and phospholipid membranes, an area of significant current interest. Focusing on antimicrobial peptides, factors affecting peptide-membrane interactions are addressed, including effects of peptide length, charge, hydrophobicity, secondary structure, and topology. Effects of membrane composition are also illustrated, including effects of membrane charge, nature of the polar headgroup, and presence of cholesterol and other sterols. Throughout, novel insights on the importance of peptide adsorption density on membrane stability are emphasized, as is the correlation between peptide adsorption, peptide-induced leakage in model liposome systems, peptide-induced lysis of bacteria, and bacteria killing.

1. Introduction

Infectious diseases account for millions of deaths worldwide each year and incur tremendous health care costs. The disease spectrum is broad and includes acute disease, such as erysipelas, sepsis, pneumonia, and numerous other infections, having a direct association to a given pathogen, as well as chronic diseases, where microbes often cause a long-standing inflammatory state. In many respects, we have reached a point for certain infections where no therapeutic agents are longer available. The well-known MRSA and vancomycin-resistant *Staphylococcus aureus* are only parts of a broader health crisis. At least three other bacterial species, all potentially life threatening, are now resistant to more than 100 different drugs. As a result of these accelerating problems with multidrug resistance, there is large current interest in antimicrobial peptides (AMPs) (1). AMPs are key components of the innate immune system, where they constitute a first line of defense against invading pathogens. AMPs can be as short as six amino acids but they average at 30 (2). Many AMPs have distinct amphiphilic characteristics with about 50% hydrophobic residues, frequently appearing in patterns of 1-2 for every 3-4 residues. The reason for this is that when the peptide forms an α-helical structure the hydrophilic residues end up on the same side of the helix normal, thereby resulting in a conformation-dependent amphiphilicity. Among secondary structures, the α-helix is the most common, found in about 30% of the AMPs (2). Another reoccurring feature in AMPs is the high frequency of cationic lysine and arginine, while anionic aspartic and glutamic acid are relatively rare. Most AMPs therefore carry a net positive charge, the average net charge being +4. Another amino acid

that stands out is tryptophan, which is 50% more common in AMPs than its general occurrence (2). The preference for these amino acids in AMPs is motivated by the specific characteristics they confer to peptide performance. For example, the cationic charge of the vast majority of AMPs is a result of the anionic nature of most microorganisms. By charge attraction, the peptides reach lytic concentration in anionic bacteria membranes. In addition, amphiphilicity is important for many AMPs in order to adsorb to and disrupt membrane bilayers, requiring hydrophobic and/or surface active amino acids (3).

Although AMPs affect bacteria in many different ways, including inhibition of cell wall, DNA, RNA, and protein synthesis, and inhibition of enzymatic activity, the main mode of action of AMPs is the disruption of bacterial membranes (1). The walls of bacteria are complex structures. For Gram positive bacteria, the wall consists of a single (protein-containing) lipid membrane surrounded by a thick peptidoglycan envelope. The wall of Gram-negative-bacteria, on the other hand, is formed by two (protein-containing) lipid membranes with a thinner peptidoglycan layer between them, and with the outer envelope of the outer lipid membrane also containing a high fraction of negatively charged lipopolysaccharides. As will be discussed further below, also the non-lipid components may interact with AMPs, and potentially reduce the membrane-disruptive activity of AMPs through competitive AMP binding, but also promote AMP action through increasing overall AMP binding and/or through a resulting osmotic deswelling of the polysaccharide moieties, causing a mechanical pressure on the lipid membrane(s). Despite this complexity, investigations with model lipid systems offer some opportunities for reaching an understanding of AMP mode of action. Using model lipid systems, several mechanisms have been proposed for AMP-induced membrane disruption (1,4,5). The classical mechanisms are based on formation of stable or short-lived membrane pores of the barrel-stave or toroidal type, or bacterial breakdown by detergent-like action **(Figure 1)**. In the barrel-stave model, peptides form transmembrane pores composed of oligomers in which peptides have adopted an amphiphilic α-helical structure with the hydrophobic part facing the hydrophobic bilayer interior. In order to form these types of pores, quite specific peptide properties are required in terms of size, helicity, and amphiphilicity, rendering this mechanism less frequent. Indeed, barrel-stave pores have only been experimentally demonstrated to occur for a few peptides, alamethicin (6) being the most well-known example. Toroidal pores, on the other hand, can be formed by a

greater variety of peptides. Prior to formation of both barrel-stave and toroidal pores, the peptide adsorbs parallel to the membrane surface (7-9). When a certain (local) concentration is reached, the peptide either inserts into the membrane, or induces a positive curvature strain in the membrane, resulting in an opening, the so-called toroidal pore. Upon further increasing the peptide concentration, or simultaneously with toroidal pore formation, two additional scenarios may take place. In one of these, higher peptide amounts on the membrane surface may eventually cause micellization in a detergent-like manner (5), although initial pore formation is not a prerequisite for this action. In a second one, the chemical potential imbalance across the bilayer due to peptide adsorption on the outer leaflet results in peptide translocation across the membrane to the inner membrane leaflet, which can take place through transient toroidal pores or without pore formation (10). In addition, peptide adsorption in the polar headgroup region causes lateral expansion of the lipid membrane, which allows relaxation of the alkyl chains and results in membrane thinning, further facilitating membrane rupture (11). Depending on the composition of the membrane, also peptide-induced phase transitions or lipid segregation may cause membrane rupture (4). Membrane lipids such as phosphatidylethanolamine (PE), an abundant component of bacterial membranes, are also sensitive to phase transitions, and experimental data have shown that peptides may induce transitions from lamellar to cubic (12) or reversed hexagonal phases (13) in PE-containing membranes. Segregation of membrane lipids, e.g., due to favorable interactions between cationic peptides and anionic membrane lipids, may also cause membrane rupture (14).

2. Effects of peptide length

Peptide length is an important parameter for the interaction between antimicrobial peptides and phospholipid membranes. In analogy to polyelectrolyte and protein adsorption, membrane binding is expected to decrease with decreasing peptide length as a result of the increased entropy penalty (per amino acid) on adsorption. For transmembrane peptide structures, there is an additional effect of peptide length, as peptides need to be able to span the thickness of the membrane (typically ≈ 40 Å) in order to stabilize the pore. Furthermore, for peptides which depend on amphiphilicity induced by secondary structure changes, there is also an effect of a decreased tendency to form ordered secondary structures (notably helices) with decreasing peptide length. Given this, decreased membrane lysis and antimicrobial effect is anticipated with

decreasing peptide length. Interesting for investigations of such effects are peptides consisting of repeat patterns, e.g., XBBXBX and XBBBXXBX (Cardin and Weintraub motifs), where X and B represent hydrophobic/uncharged and basic amino acid, respectively (15). An attractive feature of such consensus structures is that peptide length can be varied simply by increasing the number of repeat sequences, without affecting peptide charge (distribution) and hydrophobicity (distribution). In addition, since the α-helix content of these peptides is very low, length-dependent conformation effects can be neglected. Given this, such repeat sequences are ideal for investigating effects of peptide length on the interaction with bacteria and membranes. Hence, the interaction with bacteria and lipid membranes was investigated for $(AKKARA)_n$ (n=1-4) and $(ARKAAKKA)_n$ (n=1-3) peptides (16,17a). Indeed, peptide-induced killing of *Enterococcus faecalis* and *Bacillus subtilis* decreases with decreasing peptide length. In parallel, the shorter the peptide the less pronounced its induced liposome leakage, an effect related to a decreasing peptide adsorption to lipid membranes with decreasing peptide length (**Figure 2**). (Peptide adsorption in these and the other examples discussed in this review was monitored by ellipsometry, but similar data can be obtained also by other methodologies, e.g., reflectometry, neutron reflectivity, dual polarization interferometry, surface plasmon resonance, or quartz crystal microbalance (17b,c,d).

Similar effects were observed also on truncating HKH20 (HKHGHGHGKHKNKGKKNGKH) into 10-mers (HKHGHGHGKH, GHGKHKNKGK, KNKGKKNGKH) (18). Thus, while HKH20 is potent against both Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa*, the truncated 10-mers display drastically reduced bactericidal activity, irrespectively if the truncated 10 amino acid peptide is from the C-terminus, the N-terminus, or the middle segment of the original HKH20 peptide. An analogous peptide length dependence was found for liposome leakage induced by HKH20 and the truncated peptides, where all truncated peptides showed little or no liposome leakage induction for both anionic and zwitterionic liposomes. Furthermore, the truncated 10-mers all displayed significantly lower adsorption at both anionic and zwitterionic phospholipid membranes than did the native HKH20, again illustrating the relation between peptide adsorption and resulting membrane rupture. Similarly, Deslouches et al. investigated the length dependence of the antibacterial effect of a 12-residue lytic base motif peptide and found a decreased lytic activity

with decreasing peptide length (19). Note however, that there may also be considerable room for selective truncations of naturally occurring AMPs without loosing too much activity, in some cases even resulting in improved performance of the truncated variant compared to the longer endogenous peptide. For example, various studies have demonstrated that it is possible to truncate the endogenous peptide LL-37 to less than half its length and retain antimicrobial and several other functions (20). Ultimately, when truncated to become sufficiently short, however, specific membrane-rupturing capacity is lost.

3. Effects of peptide hydrophobicity

As illustrated above, high AMP adsorption is a prerequisite for potent membrane disruption. Given the anionic nature of bacteria membranes, high AMP adsorption and resulting antibacterial effect can sometimes be reached by highly charged and hydrophilic AMPs. However, many important pathogens have a relatively low electrostatic surface potential, which may be reduced or even reversed, e.g., by L-lysine modification of phosphatidylglycerol, D-alanine modification of cell wall teichoic acid, and aminoarabinose modifications in lipopolysaccharide (LPS), reducing AMP binding to Gram-positive and Gram-negative bacteria, respectively (21). Furthermore, electrostatic AMP binding is salt sensitive, and bactericidal potency of such peptides at high ionic strength limited. Alternatively, increased AMP adsorption and antimicrobial effect may be achieved by increasing AMP hydrophobicity, although this may come at a price of decreased selectivity and increased toxicity. Thus, since membrane accumulation for hydrophobic peptides is driven by poor solvency of the peptide in aqueous solution, peptide accumulation does not differentiate between membranes of different compositions, causing lysis of bacteria and human cell membranes alike. For example, peptides composed of L and K only, with a high hydrophobicity to charge ratio (e.g., KLLKLLLKLLKLLLKLLLKLLK), are even more hemolytic than the bee venom melittin (3). Thus, increased AMP hydrophobicity can only be used to some extent to boost peptide potency.

In the search for efficient and selective AMPs, increasing focus is currently placed on those of endogenous origin (20,22-25). Illustrating the effects of hydrophobicity on the membrane interactions of such peptides, we investigated CNY21 (CNYITELRRQHARASHLGLAR), a peptide derived from the C-terminal part of complement factor C3a, found to display

antimicrobial activity and low toxicity (22). In parallel, CNY21 destabilizes phospholipid membranes, an effect enhanced by increasing peptide charge and hydrophobicity (26). Thus, CNY21L (CNYITELRRQLARASLLGLAR), in which the two histidines of CNY21 have been replaced by hydrophobic leucines, results in a higher adsorption at lipid membranes, as well as in higher peptide-induced liposome leakage (**Figure 3**). In order to clarify the mechanism of peptide-induced membrane destabilization for CNY21, electrochemical methods were used to study the interaction between variants of CNY21 and dioleoyl phosphatidylcholine (DOPC) monolayers (27). Increasing the peptide hydrophobicity by substituting the two histidine residues with leucine was found to result in a deeper peptide penetration into the hydrophobic region of the DOPC monolayer, while increasing the peptide net charge from +3 to +5 by replacing the histidines by lysines arrests the peptide in the lipid headgroup region. Together, the results indicate that both hydrophobic and electrostatic interactions promote the adsorption to the membrane, while hydrophobic interactions are needed for deeper penetration. Reduction of electroactive ions (Tl^{+}, Pb^{2+}, Cd^{2+}, and Eu^{3+}) was employed to further characterise the types of defects induced by the peptides. All peptides studied permeabilize the monolayer to Tl^{+} to an appreciable extent, but this effect is more pronounced for the more hydrophobic CNY21L, which also allows penetration of larger ions and ions of higher valency. The differences observed between the different CNY21 peptides in terms of capacitance and impedance correlate well with results on peptide-induced Tl(I) permeability through DOPC monolayers. Thus, CNY21L, interacting more extensively with the hydrophobic part of the membrane, disrupts the monolayer in such a way that more Tl(I) is allowed to penetrate through the membrane and reach the mercury electrode. Tl(I), which has the smallest hydrodynamic radius (~1.3 Å) and the lowest net charge (+1) of the ions investigated, passes through the CNY21L-disrupted monolayer to a considerably greater extent than the other electroactive ions investigated. This could be an effect of either size or charge. The difference in permeability between the divalent ions Pb(II) (~2.6 Å) and Cd(II) (~5.3 Å), on the other hand, is only a matter of size. Thus, defects under these conditions are most probably larger that the size of the hydrated Pb(II) ions. However, the trivalent Eu(III), with an only marginally larger hydrodynamic radius (~3.6 Å) than that of Pb(II), does not penetrate the monolayer after addition of CNY21L, indicating that permeability through CNY21L-disrupted layers is also limited by charge repulsion. Finally, by comparing electrochemical results for DOPC monolayers with peptide-induced leakage from liposomes, i.e.,

the corresponding bilayer system, it is evident that the trends observed for CNY21, and CNY21L are similar also when an inner leaflet is present. Thus, the similar effects observed for the bilayer and monolayer systems point to a mechanism of action where formation of transmembrane structures does not play an important role (**Figure 3**).

There are also numerous other studies in the literature which demonstrate the importance of peptide hydrophobicity for AMP binding to, and destabilization of, phospholipid membranes, as well as for antimicrobial action. For example, Cornut et al. investigated peptides composed of lysine and leucine only, and found peptide-induced membrane lysis to increase with increasing peptide hydrophobicity (3). Both hydrophobicity and hydrophobic moment was furthermore demonstrated by Dathe et al. to be of critical importance for activity of KLA peptides against Gram-positive bacteria (28). Peptide hydrophobicity has also been found to be a key parameter for antimicrobial effect in a number of QSAR (quantitative structure-activity-relation) investigations (23,29).

4. End-tagging peptides with hydrophobic moieties

As an alternative to sequence-specific point mutations with hydrophobic amino acids, hydrophobic modification of AMPs may be achieved simply by attaching an acyl group to the peptide (30,31). As for antimicrobial peptides, the main target of such lipopeptides is the bacterial membrane(s). Through insertion into the membrane, lipopeptides introduce defects, leading to membrane depolarization, reduced ability for ATP synthesis, and many other detrimental effects. In analogy to hydrophobic point mutations, discussed above, increasing peptide hydrophobicity through elongation of the alkyl chain in lipopeptides results in increased membrane disruption in lipid membranes, and in increased activity against bacteria and fungi (30,31). In parallel, however, these peptides display substantial toxicity and lack of membrane selectivity, which has restricted their use to local applications, and to severe indications for which other antibiotics are ineffective, e.g., multiresistant *P. aeruginosa* infections in cystic fibrosis (30). Somewhat analogous to such lipopeptides, we therefore identified end-tagging of AMPs with hydrophobic amino acid stretches as an interesting approach to achieve high, but selective, AMP adsorption and potency, also at high ionic strength and against bacteria of low electrostatic charge density (32-34). Although a number of hydrophobic amino acids may be used as end-tags, W- and F-

based ones are particularly potent. These bulky and polarizable residues have an affinity to interfaces, and are frequently located close to the polar headgroup region in phospholipid membranes (19,35,36). Through this, W/F residues are able to insert into the membrane, acting as an anchor for the peptide, and resulting in increased bactericidal effects and salt resistance.

For example, end-tagging promoted binding of GKH17 (GKHKNKGKKNGKHNGWK) to bacteria and caused subsequent permeabilization (32). Circular dichroism showed that the peptide remained largely disordered on membrane binding, while fluorescence spectroscopy (lack of blue-shift in W fluorescence spectra) showed that W tags are located in the proximity of the membrane polar headgroup region. While permeabilization by non-tagged GKH17 was essentially non-observable, it increased strongly with the length of the hydrophobic tag. In parallel, tagged GKH17 caused release of intracellular material of bacteria, which increased with the length of the hydrophobic tag. In agreement with these findings, the binding of GKH17-WWW was higher than that of GKH17 at model dioleoylphosphatidylethanolamine/dioleoylphosphatidylglycerol (DOPE/DOPG) lipid membranes, and peptide-induced leakage from liposomes prepared from the same lipids was dramatically enhanced for GKH17-WWW compared to GKH17 (**Figure 4**). Also in parallel to bacterial killing, salt resistance of liposome leakage induction increased with the length of the hydrophobic tag. In contrast to the anionic and cholesterol-free DOPE/DOPG ("bacterial") membrane, that mimicking human cell membranes (DOPC/cholesterol) displayed lower peptide binding, also for the W-tagged peptides, as well as lower liposome leakage induction. In contrast to poorly soluble AMPs and lipopeptides with long alkyl chains, W/F end-tags thus contribute to AMP selectivity between bacterial and human membranes. For a hydrophobic residue to be able to penetrate into the phospholipid membrane, it must overcome the cohesive energy of the latter. As cholesterol condenses the phospholipid bilayer (37), end-tagged peptides become sensitive to cholesterol. Particularly for bulky groups such as W and F, which require substantial area expansion (36), tag insertion into membranes containing cholesterol becomes an energetically costly process. Consequently, the adsorption of tagged peptides is reduced at cholesterol-containing membranes, as is membrane-induced leakage induction of the corresponding liposomes (32). This difference in membrane interactions in the presence of absence of cholesterol, together with that due to the charge difference between the anionic ($z \approx -30$ mV) and

zwitterionic (z ≈ -10 mV) membranes used, contributes to the selectivity between bacteria and human cells observed. Interestingly, oligomerization seems to play a key role for lipopeptides containing moderately to long alkyl chains (31), but not for the W/F-tagged AMPs investigated (32).

The interplay between electrostatic and hydrophobic interactions for end-tagged peptides was further investigated for model cationic sequences (38). In doing so, heptamers of lysine (K7) and arginine (R7) were investigated and found to be lytic against *E. coli* at low ionic strength. In parallel, both peptides adsorbed to bilayers formed by *E. coli* phospholipids, and caused leakage in the corresponding liposomes. K7 was the more potent of the two peptides in causing liposome leakage, although the adsorption of this peptide on *E. coli* membranes was lower than that of R7. The bactericidal effect, liposome lysis, and membrane adsorption were all substantially reduced at physiological ionic strength. When a tryptophan pentamer tag was linked to the C-terminal end of these peptides, substantial peptide adsorption, membrane lysis, and bacterial killing was observed also at high ionic strength, and also for a peptide of lower cationic charge density (KNKGKKN-W5). Strikingly, the order of membrane lytic potential of the cationic peptides investigated was reversed when tagged, suggesting that tagged and untagged peptides act by different lytic mechanisms, which to some extent counterbalance each other. Thus, while the untagged peptides act by generating negative curvature strain in the phospholipid membrane, the tagged peptides cause positive curvature strain. The tagged heptamer of arginine, R7W5, was the best candidate for *E. coli* membrane lysis at physiological salt conditions and proved to be an efficient antibacterial agent.

5. Effect of peptide charge

Since bacterial membranes are rich in anionic phospholipids, while those of human cells are dominated by zwitterionic lipids, electrostatics plays an important role in AMP-membrane interactions, and an overwhelming majority of AMPs carry a net positive charge (2). Similarly, increasing the AMP positive charge constitutes a way to increase peptide adsorption to negatively charged lipid membranes, as well as membrane lysis. Importantly, however, electrostatic interactions are screened at high ionic strength and in the presence of multivalent ions (i.e., at physiologic conditions). Pure electrostatic interactions are also not efficient for bacteria and other

pathogens displaying low surface electrostatic potential, and also sensitive to inactivation through bacterial release of anionic AMP scavengers (e.g., glucose aminoglycan or extracellular polysaccharides) (21). Finding a good balance between electrostatic and non-electrostatic interactions is therefore paramount for identifying AMPs of therapeutic interest. Staying with the CNY21 example discussed above, both peptide adsorption and resulting membrane rupture by CNY21-variants increased with increasing peptide positive charge, and was completely lost on elimination of all peptide positive charges (26). In parallel, elimination of the peptide positive net charge drastically reduced bactericidal effect on both *P. aeruginosa* and *B. subtilis*. Impedance spectroscopy and electrochemical investigations demonstrated that increased positive charge of CNY21 resulted in arrest of the peptide in the outer part of the polar headgroup region of the phospholipid membrane, and that the defects formed an electrostatic barrier for cations to permeate peptide-perturbed membranes, respectively (27). That electrostatically driven adsorption represents the main driving force for membrane disruption in these systems was demonstrated also by a drastic reduction in both liposome leakage and peptide adsorption with increasing ionic strength, and that this salt inactivation can be partly avoided by increasing the peptide hydrophobicity.

Effects of electrostatic interactions were further investigated using peptides containing titratable histidine groups. As discussed above, peptides composed of multiples of the Cardin and Weintraub sequences AKKARA and ARKKAAKA are antimicrobial. Replacement of lysine and arginine by histidine in these peptides to obtain $(AHHAHA)_4$ (AHH24:1) and $(AHHHAAHA)_3$ (AHH24:2) completely abrogates their antimicrobial activities at neutral pH (39), i.e., above the pKa of histidine (≈6.0) (**Figure 5**). However, the antibacterial activity of the latter peptides against Gram-negative (*E. coli*, *P. aeruginosa*) and Gram-positive (*B. subtilis* and *S. aureus*) bacteria, as well as the fungus *C. albicans*, was restored at acidic conditions (pH 5.5) (39). In parallel, fluorescence microscopy and FACS analysis showed that peptide binding was significantly enhanced at pH 5.5, while electron microscopy analysis of peptide-treated bacteria, paired with analysis of peptide effects on liposomes, showed that the peptides exerted membrane breaking effects only at acidic pH. Also in parallel, AHH24 peptides displayed lower adsorption to phospholipid membranes at pH 7.4 than did the corresponding peptides with R and K replacing H (17). Similar pH-dependent antimicrobial activities were demonstrated for the histidine-rich

peptide histatin 5 and for peptides derived from two endogenous proteins having histidine-rich regions, i.e., high molecular weight kininogen and histidine-rich glycoprotein. The results demonstrate that the presence of an acidic environment is important for the antimicrobial activity of histidine-rich antimicrobial peptides. Analyzing peptide charge showed that peptides displaying antimicrobial activity at pH 7.4 all carry a high net positive charge at this pH, ranging from +5.3 for histatin 5, to +12.0 for AKK24/ARK24. For peptides displaying no antimicrobial activity at neutral pH, the net positive charge at pH 7.4 ranged from -1.8 (GHG21) to +2.3 (GGH20). Notably, a gain in net positive charge at pH 5.5 (ranging from +4.6 to +10.8) correlated well with an increase in antimicrobial activity (39). (Here, one may note in parenthesis, that this pH-dependent membrane lysis could potentially be used for endosomal escape, e.g., in DNA or siRNA delivery, in a similar way observed for titrating polysaccharides such as polyethyleneimine.)

Effects of peptide charge were also investigated for variants of the human kininogen-derived peptide HKH20 (HKHGHGHGKHKNKGKKNGKH) (18). Due to its high net charge (+6) and relatively hydrophilic nature (-0.77 on the Eisenberg scale), electrostatic interactions play a central role in the interaction between the random coil HKH20 and lipid bilayers. Thus, leakage induction is significantly higher for anionic than for zwitterionic liposomes, and the capacity to cause liposome leakage is strongly decreased at high ionic strength. The latter effect is seen also in bacterial killing, displaying >95% reduction in bactericidal effect at 150 mM NaCl. In parallel, adsorption of HKH20 is significantly higher to anionic than to zwitterionic bilayers, while HKH20 adsorption is very low for both lipid membranes at high ionic strength. These observations clearly indicate that electrostatics dominates the interaction between HKH20 and lipid bilayers. Similarly, Makovitzki and Shai investigated the pH dependence of lipopeptides with dodecanoic acid-modified 12-mer peptides of the type LXXLLXXLLXXL, where X is H, K or R (40). While the K- and R-containing peptides displayed little pH dependence, an H-containing one showed pronounced pH dependence. This H-containing peptide did not display any antifungal properties at pH 7.4 (contrary to the K- and R-containing peptides), but was active at pH 5.5. Analogously, the peptide induced liposome leakage at the lower pH but not at the higher, an effect related to a higher peptide adsorption at lipid membranes at the lower pH. From these and numerous other studies (e.g., 1,5,23,29), there is little doubt that when increasing the

positive peptide charge from close to zero to a high value, peptide adsorption to negatively charged lipid surfaces increases, as do membrane lytic effects in both liposomes and bacteria. What results obtained with the highly charged HKH20 show, however, is that there is a limit to these charge-induced effects at sufficiently high peptide charge density due to a decreased peptide adsorption (18). Thus, at pH 5.5 (z_{net}=+12), both peptide adsorption and membrane disruption are lower than those at intermediate peptide charge density (z_{net}=+6) at pH 7.4, but nevertheless significantly higher than for the corresponding uncharged peptide variants (z_{net}=0). An analogous decreased bactericidal activity at very high peptide charge was found by Giangaspero et al. (41). Similarly, both polyelectrolytes and polyampholytes display decreased adsorption at high macromolelecule charge densities, primarily due to the electrostatic penalty from effectively reducing the intramolecular distance between charges on adsorption (42).

Apart from electrostatic effects related to charge content or fraction of charged histidine residues, there are also finer effects of the nature of the cationic residues. In analogy to membrane perturbing effects of longer polymers of lysine and arginine, leakage can be induced in *E. coli* liposomes by heptamers of lysine or arginine at low ionic strength, while this lytic activity is substantially reduced at physiological ionic strength. Also peptide adsorption to the corresponding supported bilayer demonstrates that electrostatic interactions play an important role, as adsorption is drastically reduced at high ionic strength. Despite this obvious importance of electrostatic effects, there are also striking differences between the structurally closely related R7 and K7 peptides, R7 displaying a significantly higher adsorption to the anionic membrane. Similarly, polyarginine displays higher binding affinity to anionic surfactants (43), nucleotides (44), and anionic microgels (45). Despite the more extensive adsorption of R7 to E. *coli* lipid membranes, R7 caused lower leakage induction than K7 in the corresponding liposomes. Although arginine has a higher pKa than lysine (~12.5 vs. ~10.5), which may also contribute to its higher adsorption, this only explains part of the behavior. Thus, the delocalized charge of the guanidinium group of arginine, although highly water soluble, can be made lipid soluble through ion pairing with anionic lipids, while amino groups do not show this behavior (46). As a result of this, short homopolymers of arginine are efficient cell-penetrating peptides, able to translocate across cell membranes, an ability not shared by corresponding lysine peptides (47). Another factor causing R7 to adsorb to a higher degree than K7 is its greater distance between peptide

backbone and the cationic charges, resulting in an increased effective reach. When polycationic peptides adsorb to partially anionic membranes, an accumulation of anionic lipids in the proximity of the adsorbed peptide is expected. This results in an entropy penalty that is less profound for a peptide where the cationic groups are more separated, contributing to higher adsorption levels for R7. The proximity of the cationic groups on K7 is instead likely to generate a more profound demixing of the anionic and zwitterionic lipids. This effect, combined with lower peptide flip-flop frequency, leads to a higher curvature strain on the outer monolayer, contributing to the higher leakage induction by K7.

6. Effect of peptide topology and secondary structure

Given the frequent observation of ordered secondary structures accompanying AMP binding to lipid membranes, as well as the biological importance of cyclic structures for some classes of AMPs (notably defensins), there has been some interest in the effect of peptide topology for AMP interactions with lipid membranes. Addressing this issue, we investigated effects of peptide topology for linear and cyclicized C-HKH20-C (18). Since this peptide is characterized by a disordered random coil conformation, no interference of secondary structure effects accompanies the cyclization, hence the peptide is well suited for studies of effects of peptide topology. Investigating Gram-positive *S. aureus* and Gram-negative *E. coli*, the antibacterial potency of the cyclic peptide variant was found to be lower than that of the linear one, an effect observed both at pH 7.4 and 5.5. In parallel, liposome leakage induced by the cyclic peptide is significantly lower than that of the linear variant, an effect related to a lower adsorption of this peptide. Due to the connectivity of the peptide ends for the cyclic peptide, its conformational freedom is smaller than that of the linear variant. Consequently, the conformational entropy loss on adsorption is smaller for the cyclic peptide than for the linear one, which should contribute to a higher adsorption density for the cyclic peptide (48). The observation of the reverse behaviour shows that other factors are important in determining the peptide adsorption as well. Most likely, the bulky nature of the cyclic peptide prevents the peptide from penetrating into the lipid headgroup region, and hence looses some of the hydrophobic driving force for adsorption and membrane defect formation. Similarly, Krishnakumari et al. (49) and Unger et al. (50) found cyclic peptide variants to be less potent in antimicrobial or liposome leakage assays than the corresponding linear peptide variants. However, the effect of peptide cyclization seems to be somewhat peptide-

specific regarding bactericidal effects, as also decreased hemolysis but retained antimicrobial activity have been found on cyclization of tachyplesin I (51). Furthermore, Unger et al. noted a decrease in antimicrobial potency of a magainin 2 analogue on cyclization, but an increased potency of a melittin analogue (50). However, in terms of liposome leakage induction, also the latter investigation showed the cyclic variant to be less effective than the corresponding linear peptide variant for both the magainin 2 analogue and the melittin analogue. Furthermore, it was found that the main effect of cyclization in liposome systems was a decreased peptide binding to the phospholipid membrane (49). Overall, therefore, these findings on the effects of peptide cyclization are in line with those found for HKH20.

The ability of AMPs to form amphiphilic ordered structures (notably α-helices) has been found to correlate to the ability of such peptides to disrupt lipid membranes and to kill bacteria (1,5-9,23,52). For some peptides, e.g., melittin, alamethicin, magainin 2, and gramicidin A, transmembrane structures have been reported, although controversy regarding these exists for some of these systems. More frequently, peptides undergoing helix induction are characterized by helices oriented parallel to the lipid membrane. In analogy to structurally disordered peptides, membrane disruption for such peptides is obtained by other mechanisms, e.g., induction of a negative curvature strain, membrane thinning, or local packing defects (see above). A peptide displaying pronounced helix induction on membrane interaction is LL-37 (LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES). LL-37 displays strong antibacterial effects, and also neutralizes lipopolysaccharide, stimulates immune cells, and promotes re-epithelization and wound closure (20). Challenges with LL-37 from a therapeutic perspective include non-negligible cytotoxicity, as well as susceptibility to proteolytic degradation. This has sparked an interest in LL-37 variants which are proteolytically more stable, and/or less toxic, than LL-37 (20,53). Also along this line, we previously identified GKE21 (GKEFKRIVQRIKDFLRNLVPR) as a promising peptide which maintains the bactericidal potency and LPS-binding capacity of LL-37, but which displays lower toxicity (25). An interesting feature of GKE21 is its potential ability to form a nearly perfectly amphiphilic (idealized) helix, where hydrophobic/nonpolar and polar/charged residues are localized on opposite sides (54). Using GKE21 as a template, effects of single valine substitutions (V->dV or V->P) to destabilize the helix were therefore addressed. GKE21 displayed moderate helix

induction in buffer, which increased on interaction with phospholipid membranes. Substituting either of the two valines in GKE21 with either proline or D-valine resulted in helix destabilization, while peptide isoelectric point, net charge at pH 7.4, and mean hydrophobicity remained unchanged. Although quantitatively minor, the decreased tendency for helix formation in GKE21 (V->P, V->dV) resulted in a lower induced (helix-related) amphiphilicity, and correlated to lower peptide adsorption at supported phospholipid membranes, as well as to decreased peptide-induced liposome leakage, particularly at high electrolyte concentration where conformation-invariant electrostatic interactions are screened. (Peptide secondary structure in the examples discussed was obtained from circular dichroism spectroscopy, but similar data may be obtained also by IR or NMR spectroscopy.) Accordingly, bacterial killing was reduced for the conformationally destabilized peptides, indicating that even minor changes in induced peptide amphiphilicity may be of relevance for the bactericidal properties of this type of antimicrobial peptides. These effects were further evaluated for EFK17 (EFKRIVQRIKDFLRNLV), again forming a perfectly amphiphilic helix (in the idealized case), with all polar/charged residues on one side, and all unpolar/hydrophobic residues on the other (**Figure 6**) (55). Thus, formation of an ordered helix is accompanied with an induced amphiphilicity. In an effort to simultaneously investigate proteolytic stability of EFK17, the peptide was modified by four d-enantiomer or W substitutions at known protease cleavage sites, as well as by terminal amidation and acetylation. The d-enantiomer substitutions rendered the peptides indigestible by all four proteases investigated, however those peptides displayed minor antimicrobial potency. The similarly proteolytically stabilized W- and end-modified peptides, on the other hand, showed an increased bactericidal potency compared to the native peptide sequence. As with bactericidal potency, the latter peptides were at least as potent as the native EFK17 peptide in causing liposome leakage, while the d-enantiomeric variants were significantly less lytic. Thus, peptide-induced liposome leakage correlated with bactericidal potency, as did peptide adsorption to the lipid membrane. Importantly, higher membrane-induced helicity correlates to a higher adsorption density, a reasonable finding given the amphiphilic nature of the idealized helix formed in these systems (**Figure 6**). For example, terminal acetylation/amidation was found to increase peptide helicity, an effect due to stabilization of the helix by hydrogen bonding to an unsatisfied main-chain NH or CO group, respectively. In parallel, peptide adsorption, liposome leakage, and antimicrobial potency were enhanced for the acetylated/amidated peptides. This is in line with previous

findings, where increased helicity in antimicrobial peptides has been found to correlate to increased antibacterial potency (56). Similarly, W-substitutions significantly increased peptide helicity and peptide amphiphilicity, thus resulting in higher peptide adsorption, increased peptide-induced liposome leakage, and increased antibacterial potency. On the contrary, the d-enantiomer substitutions resulted in substantially lower adsorption and liposome leakage induction, an effect due to the very limited helix induction, and resulting amphiphilicity, both in solution and after adsorption to the lipid membrane. Internal d-enantiomer substitutions and loss of antibacterial activity have previously been coupled with loss of helicity (57). Also other means for reducing helicity, such as proline substitutions, have been found to reduce peptide interaction with phospholipids membranes (58), and decreasing helicity by inclusion of d-enantiomers or proline in amphiphilic antimicrobial peptides has been correlated with decreased bactericidal and cytotoxic effect (41). Destabilizing helixes for antimicrobial peptides has therefore been suggested as a way to reduce cytotoxicity (23,59). As demonstrated for EFK17, however, helix destabilization may ultimately result also in elimination of peptide adsorption, membrane lysis, and antimicrobial effect, at least for peptides forming strongly amphiphilic helices.

7. Selectivity in peptide interaction with lipid membranes

A key issue for successful development of AMP therapies is that selectivity is reached, such that bacteria and other pathogens are efficiently killed, whereas membranes of human cells are left unperturbed. Human cell membranes and bacterial membranes have similar functions but differ in composition. For example, cholesterol is present in human cell membranes, constituting up to 45% of the total lipids, while it is absent in bacterial membranes, or replaced by ergosterol in fungi. In addition, there are considerable differences in phospholipid composition. For example, in the membrane of red blood cells, phosphatidylcholine (PC) and sphingomyelin (SM) are abundant in the outer layer, while the inner leaflet mainly contains amino lipids (phosphatidylethanolamine (PE) and phosphatidylserine (PS)). These lipids are zwitterionic (except for PS), rendering the outer part of the membrane uncharged. On the contrary, the outer membrane of bacteria is rich in anionic lipids. For example, *E. coli* membranes are typically composed of about 91% PE, 3% phosphatidylglycerol (PG), and 6% cardiolipin, while the cytoplasmic membrane contains 82% PE, 6% PG, and 12% cardiolipin (61). *S. aureus* membranes, on the other hand, contain PG lipids only, typically of the order 36% PG, 7%

cardiolipin, and 57% lysylPG (62). In addition, both Gram-positive and Gram-negative bacteria contain peptidoglycan, while Gram-negative bacteria contain also negatively charged LPS (up to 50% of the outer membrane), and Gram-positive bacteria contain also teichoic acid. Despite these discriminators in membrane composition, obtaining high selectivity between bacteria and human cells remains a challenge. Given this, significant efforts have been directed to identifying selective AMPs through screening for AMPs from various species, and through use of combinatorial libraries, in combination with quantitative structure-activity relationships (QSAR) for AMP identification (1,2,5,23,29). Another opportunity in this context involves identification of AMPs (potentially) generated through proteolytic degradation of endogenous proteins during bacterial infection. Using the latter approach, selective AMPs have been identified, originating from the human complement (22), contact (24), and coagulation (63) systems, as well as from serum (64), matrix (65), or growth factors (66a). Although the necessity to understand the origin of membrane selectivity has thus been partly circumvented through these lead identification and optimization approaches, it is still important to attempt to understand these rather complex issues better. While membrane selectivity is less understood than properties effecting AMP lytic potency in general, some of the major effects are relatively well defined, at least for model lipid membranes. A couple of examples of this are provided below.

Effects of membrane charge

Although differing considerably between systems, some AMP selectivity is frequently observed between bacteria and human cells, and bacteria-mimicking liposomes are often found to be more susceptible to peptide-induced rupture than liposomes mimicking human cells. Staying with one of the examples discussed above (32), AMPs end-tagged with W/F oligomers were found to display good functional selectivity, having potent effects against a range of Gram-positive and Gram-negative bacteria, but at the same time displaying low toxicity to human cells. In parallel, GKH17-WWW potently caused leakage of bacteria-mimicking DOPE/DOPG liposomes, while peptide-induced liposome leakage was substantially lower for zwitterionic DOPC liposomes. Furthermore, DOPC membranes displayed substantially lower adsorption of GKH17-WWW. Consequently, the charge difference between DOPG/DOPE ($z \approx -30$ mV) and DOPC ($z \approx -10$ mV) membranes most likely contributes to the selectivity observed. The situation is quite complex, however, as there are also many other possible origins to the observed selectivity

between bacteria/fungi and human cells. Apart from the cholesterol effect discussed below, GKH17-WWW also displays pronounced binding to bacterial LPS, which may well result in a high binding of this peptide to Gram-negative bacteria. LPS, a non-lipid membrane component, may therefore contribute to membrane selectivity, either through is (charged) polysaccharide moieties, or through its hydrophobic lipid A domain. Thus, charge interactions between peptides and membrane phospholipids are unlikely to provide the sole basis for membrane selectivity.

Investigating effects of membrane charge further, we performed a series of studies on peptide binding to zwitterionic DOPC/cholesterol and anionic DOPC/DOPA/cholesterol membranes. Peptides dominated by random coil conformation were selected for these studies, eliminating any effect of membrane charge on peptide secondary structure after membrane binding. Furthermore, dioleoyl lipids were used throughout in order to suppress melting transitions and phase separation. Together, this facilitates studies of electrostatic interactions without complicating interference from these other factors. As expected, the higher charge of the DOPA-containing membranes ($z \approx -7$ mV and -35mV for the zwitterionic and the anionic system, respectively) results in higher adsorption of cationic peptides, observed both for CNY21 variants (26), repeat consensus peptides (17), and HKH20 (18). Also quantitatively, the importance of electrostatic interactions is illustrated by the roughly 3-fold higher peptide adsorption obtained for the anionic membranes. For highly charged peptides, such as HKH20 (18) and AKK24 (17), this higher peptide adsorption is accompanied by a greater peptide-induced liposome leakage for the anionic system. The higher affinity for anionic lipid components may also be used for selective binding of peptides to oxidized lipids in atheroschlerosis contexts, as elegantly demonstrated by Epand et al. (66b). In addition, preferential binding to negatively charged lipids in mixed membranes may cause domain formation (66c). For peptides of lower net charge (e.g., CNY21 variants), relatively modest differences are observed between leakage induction in the anionic and zwitterionic liposomes, despite a much higher peptide adsorption to the anionic membrane (26). Thus, and as discussed elsewhere in this paper, peptide adsorption density is an important, but not the only, determinant for peptide-induced membrane rupture. From these studies, also another couple of general observations can be made. First, the ranking between different peptides in terms of both peptide adsorption and peptide-induced liposome leakage is generally the same, largely independent of membrane charge, which means that there is some freedom in the choice of lipid

composition for studies of peptide-membrane interactions, e.g., facilitating lipid membrane designs which allow stable monolayers, bilayers, and liposomes to be formed, systems where domain formation can be suppressed, etc. This is supported by the finding that also for the seemingly non-biological DOPC/cholesterol and DOPC/DOPA/cholesterol, there is a good correlation between liposome leakage and bacterial killing (17,18,23-26,32,39,54,55). Thus, although quantitative aspects may differ depending on liposome composition, a range of liposome compositions seem to be able to offer biologically relevant information on the ranking in terms of membrane rupture between different peptides.

In order to further clarify effects of the phospholipid polar headgroup, we investigated peptide-induced membrane binding and resulting membrane rupture for the bee venom peptide melittin (NH_2-GIGAVLKVLTTGLPALISWIKRKRQQ-$CONH_2$). Interesting in this context is that melittin displays a reversed selectivity, targeting mammalian cell membranes rather than those of bacteria, still sharing important features with a number of AMPs, e.g., the magainins and cecropins. Numerous aspects of melittin interaction with liposomes have been investigated, including effects of length and saturation of the aliphatic chain of the phospholipid, effects of cholesterol and other sterols, effects of varying lipid packing parameters, and the presence of large polar headgroups (67). Primarily, studies on headgroup effect on bilayer-melittin interaction have focused on PC, PC, PE, PS, and PG. From these and other studies, it has been found that anionic lipid headgroups confer higher resistance to liposomes against melittin-induced leakage than zwitterionic ones (67). Results from Strömstedt et al. for phosphatidylinositol- (PI-) and phosphatidic acid- (PA-) containing liposomes support these findings and show that both these anionic lipids hinder melittin-induced liposome leakage (68). However, the peptide/lipid ratio required to induce 50% leakage is significantly higher for PA-containing liposomes than for PI-containing ones of the same molar ratio of anionic lipid (and comparable z-potential), an effect increasing with molar fraction of the anionic phospholipids. As expected, the adsorption of the cationic melittin at zwitterionic PC is significantly lower than that at observed the anionic PA and PI bilayers. However, quenching of the single melittin tryptophan residue by depth-specific doxyl groups shows that melittin penetrates deeper into the bilayer in the case of PC liposome compared to the PA-containing system (similar information of localization depth would be accessible also by NMR and neutron reflectivity (17d)). In the case of PI, melittin does not

penetrate into the bilayer to the same extent as for PC, but more so than for PA. Decreasing the electrolyte concentration in the case of PI yields results more comparable to PA, i.e., melittin penetrates the lipid bilayer to a smaller extent than observed in the high salt system. The decreased melittin penetration into the lipid bilayer on decreasing the ionic strength for PI-containing liposomes is manifested by a higher peptide concentration needed to induce leakage. Also from a leakage perspective, therefore, decreasing the electrolyte concentration for this liposome system renders it more similar to PA. Together, these findings show that increasing the membrane negative charge leads to increased peptide adsorption but also to increased membrane tolerance, the latter due to melittin being electrostatically arrested closer to the interface of the lipid bilayer. The presence of an additional hydration repulsion through inclusion of an inositol residue increases the lytic properties of melittin by balancing the electrostatic attraction and thus relaxing the arrest at the bilayer surface seen with the acid headgroup. This relaxation can be largely eliminated by reducing the ionic strength. The findings demonstrate that properties of the lipid polar headgroup, other than simply anionic or zwitterionic, are important in peptide-membrane interactions.

Effects of cholesterol and other sterols

Sterols are found in a wide range of membranes from various species. Thus, ergosterol and lanosterol are present in the membranes of fungi, protozoa, and insects, cholesterol is a significant constituent in membranes in the rest of the animal kingdom, while prokaryotes such as bacteria lack sterols all together. Although these sterols are structurally quite similar, their relative effects on membranes have been found to differ. Cholesterol is particularly potent in increasing lipid order in membranes while maintaining fluidity, and provides large mechanical coherence to membranes (37). As cholesterol condenses the phospholipid bilayer, peptide adsorption and penetration become sensitive to cholesterol. Particularly for bulky groups such as W and F, which tend to locate in an orientation parallel to the lipid membrane surface, and thus require substantial area expansion, insertion into membranes containing cholesterol becomes an energetically costly process. Examples of peptides sensitive to the presence of cholesterol are therefore those containing oligotryptophan residues. Indeed, lower peptide-induced liposome leakage, as well as lower peptide adsorption for DOPC/cholesterol than for DOPC, was

previously demonstrated for GKH17-WWW (32) (**Figure 4**). Hence, the presence of the bulky W/F tags not only promotes antimicrobial activity, but also contributes to membrane selectivity.

Selectivity between membranes containing cholesterol and other sterols is also of therapeutic interest. For example, like human cells, fungi are eukaryotic cells with sterol-containing membranes. While the former are based on cholesterol, the latter are formed by ergosterol. Identifying peptides selective for ergosterol-containing membranes may therefore represent a means to identify simultaneously antifungal and low-toxic therapeutics. In this context, we previously investigated histidine-rich glycoprotein (HRG) and smaller peptide sequences thereof (65). HRG exerts potent antifungal activity at low pH, mediated via its histidine-rich region, as do small peptides originating from this region. HRG was furthermore found to protect against systemic *Candida* infection, while electron microscopy demonstrated that HRG caused membrane breaks in *Candida* cells and release of cytoplasmic components. In parallel, HRG was found to disrupt liposomes. Notably, ergosterol-containing liposomes, mimicking fungal membranes, were more sensitive than cholesterol-containing ones, mimicking mammalian membranes. Through this selectivity between ergosterol-containing and cholesterol-containing membranes, HRG is able to display potent antifungal effects, at the same time as it displays low toxicity toward human cells. These results are in agreement with previous findings that ergosterol, with only marginal membrane-condensing properties compared to cholesterol, confers less stability to phospholipid membranes (37).

8. Summary

Although AMPs affect bacteria in numerous ways, e.g., interfering with cell wall synthesis, inhibition of DNA, RNA, and protein synthesis, and blocking enzymatic activity, their main mode of action is the disruption of bacterial membranes. Consequently, there is generally good correlation between bacteria killing, bacteria lysis, liposome lysis, and peptide adsorption to lipid membranes, providing studies with model lipid systems some relevance translating also to the biological situation. As demonstrated in the present overview, numerous parameters are of importance for localizing AMPs and other amphiphilic peptides at the membrane, including peptide length, charge (distribution), hydrophobicity (distribution), secondary structure, and topology, which may be used to tune also other aspects of peptide-membrane interactions

important to membrane lysis, e.g., the degree of peptide penetration to the membrane interior. Peptide design based on these and other descriptors facilitates identification of AMPs with high potency, also for challenging pathogens and conditions, while still displaying limited toxicity. Such designs may also be used to improve peptide performance in other aspects, e.g., increasing proteolytic and/or chemical stability, or facilitating efficient bioanalysis or low cost of goods in a drug development context. Although microbial killing remains the main focus for AMPs, they offer also other terapeutic opportunities, e.g., related to facilitating uptake by biomacromolecular drugs (i.e., as cell-penetrating peptides), or in the context of mitochondria membrane disruption for promoting apoptosis and reducing chemoresistance development in cancer therapy. In the context of AMP drug development, it is also worth noticing, that while there are presently no AMP drugs on the market, a couple of related lipopeptides are, where they exert potent action on a range of antibiotics-resistant bacteria. There are also a couple of AMPs that have undergone Phase III clinical trials, and about half a dozen AMPs presently undergoing Phase II clinical trials. Time will tell if any of these will have what it takes to make it to the market, and if so, what their performance will be in a broader and more long-term clinical use. In parallel, there is a need for increased focus on the interaction of AMPs and other amphiphilic peptides also with non-lipid membrane components (e.g., LPS, teichoic acid, and peptidoglycan), but also with more complex structures, including toll-like receptors. Such investigations may contribute to an increased understanding of the interplay between amphiphilic peptides and biological regulatory systems, including the coagulation and immune systems, e.g., related to immunomodulatory, anti-inflammatory, or anticancer action of such peptides.

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Figure Captions

Figure 1. Schematic illustration of different mechanisms for AMP-induced membrane destabilization.

Figure 2. (a) Antimicrobial activity of $(AKKARA)_n$ ($1 \leq n \leq 4$) peptides against *B. subtilis* determined by radial diffusion assay (RDA) at a peptide concentration of 100 μM. In RDA, peptides are placed in wells in agar gels containing confluent microbes. On radial diffusion, the peptide will kill bacteria and result in a clearance zone containing disintegrated microbes. Thus, the higher the diameter (d), the higher the growth inhibition, i.e., the more potent the peptide. Shown also is peptide-induced leakage in anionic DOPA-containing liposomes (b) and AKK6 and AKK24 adsorption to the corresponding lipid membrane (c), all in 10 mM Tris, pH 7.4. (17)

Figure 3. (a) Schematic illustration of the mono- and bilayers employed (not to scale), and permeability induced by CNY21 and its hydrophobic variant CNY21L through DOPC mono- and bilayers, investigated by Tl(I) reduction and carboxyfluorescein release from liposomes, respectively, at a peptide concentration of 0.8 μM. (b) Reduction of the electroactive ions Tl(I), Pb(II), and Cd(II) at DOPC-coated Hg-electrode after addition of different CNY21L concentrations, expressed as the selectivity ratio, i.e., the ratio between the reduction current after peptide addition and that for pure mercury at -0.6 mV. (27)

Figure 4. (a) Effect of end-tagging GKH17 with WWW on antibacterial potency against *S. aureus*. Shown also is the effect on the W-tagging on peptide adsorption to lipid membranes (b), and peptide-induced liposome leakage (c), all in 10 mM Tris, pH 7.4. (32)

Figure 5. (a) Antimicrobial activity of AKK24 and AHH24:1 against a number of bacteria strains determined by radial diffusion assay (RDA) at pH 7.4 and 5.5 and a peptide concentration of 100 μM. The higher the diameter (d), the more potent the peptide. Shown also is the corresponding peptide-induced leakage in anionic DOPA-containing liposomes (b) and peptide adsorption to the corresponding lipid membrane (c). (17,39)

Figure 6. (a) Helical weel projection of EFK17 with enantiomeric (dF or dI) and tryptophan (W) substitutions outlined. Black and white circles represents hydrophilic and hydrophobic residues, respectively. (b) Helix content of acetylated-amidated EKH17 (-a), and the corresponding d-enantiomer (-d/a) and W-substituted (-W/a) peptides. Measurements were performed in 10 mM Tris buffer, pH 7.4, with or without additional 150 mM and liposomes (100 mM lipid). Shown also is the adsorption of these peptides at the corresponding lipid membrane (c) and resulting liposome leakage (d) in 10 mM Tris, pH 7.4. (55)

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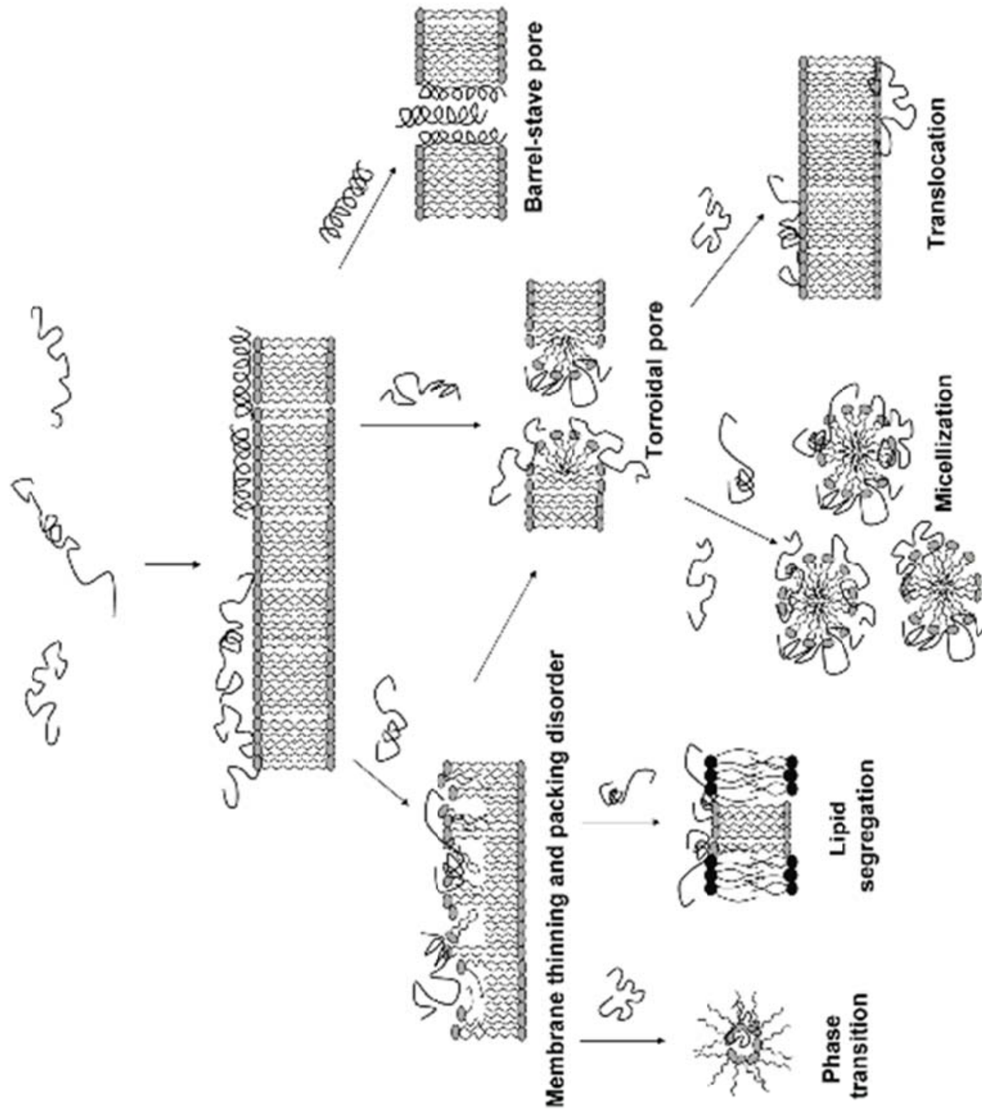

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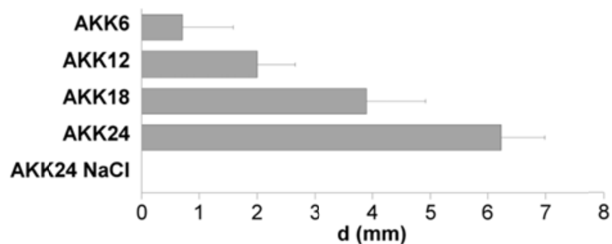

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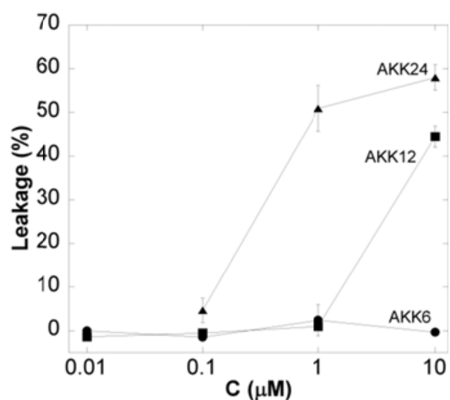

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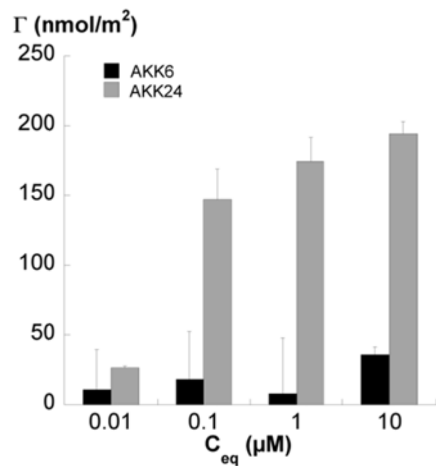

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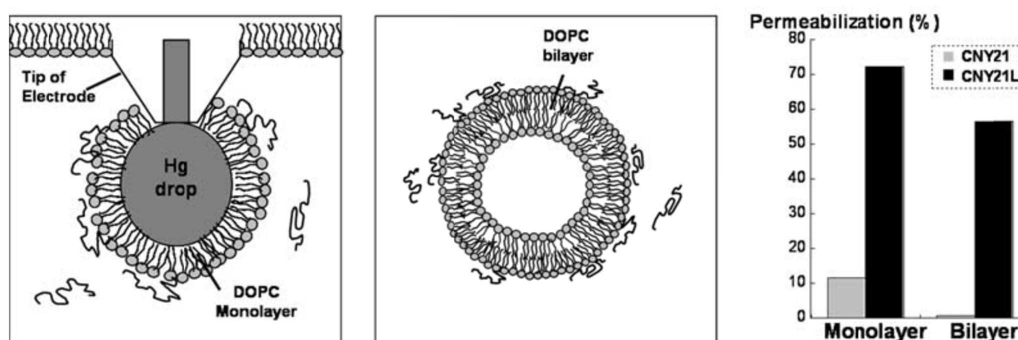

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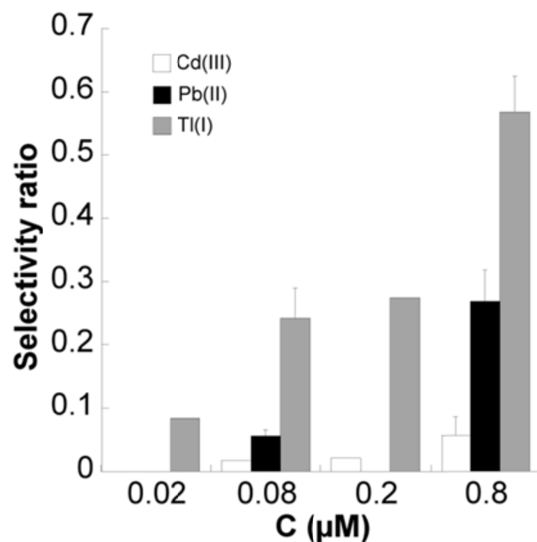

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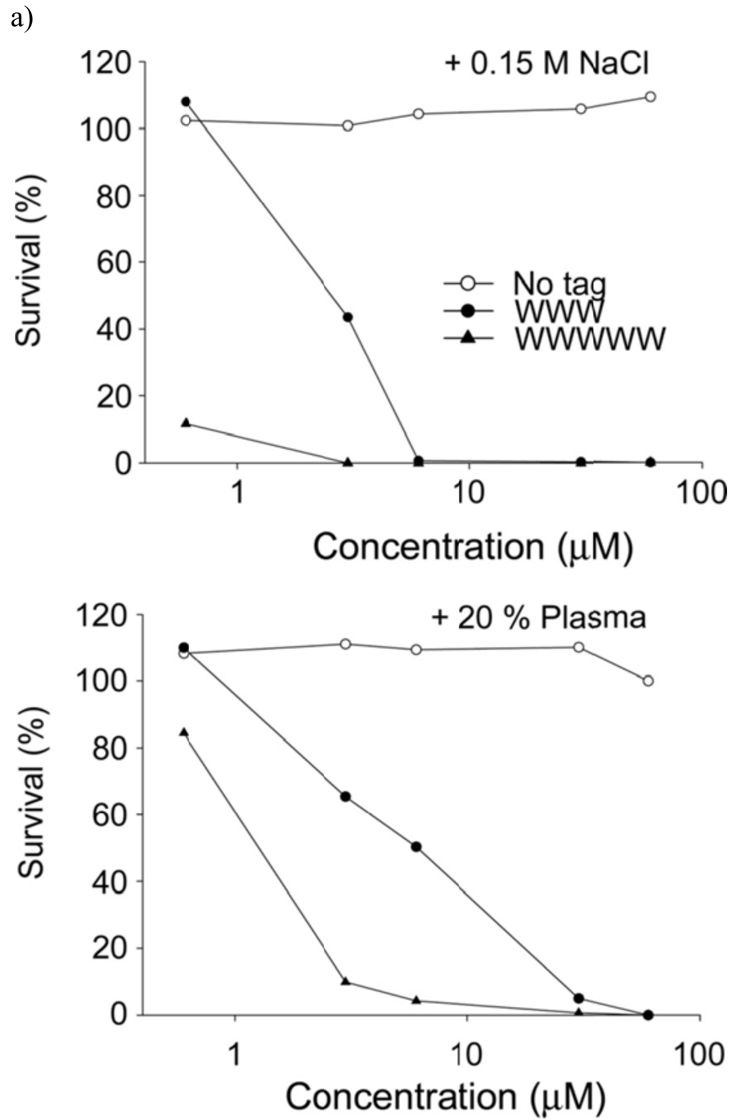

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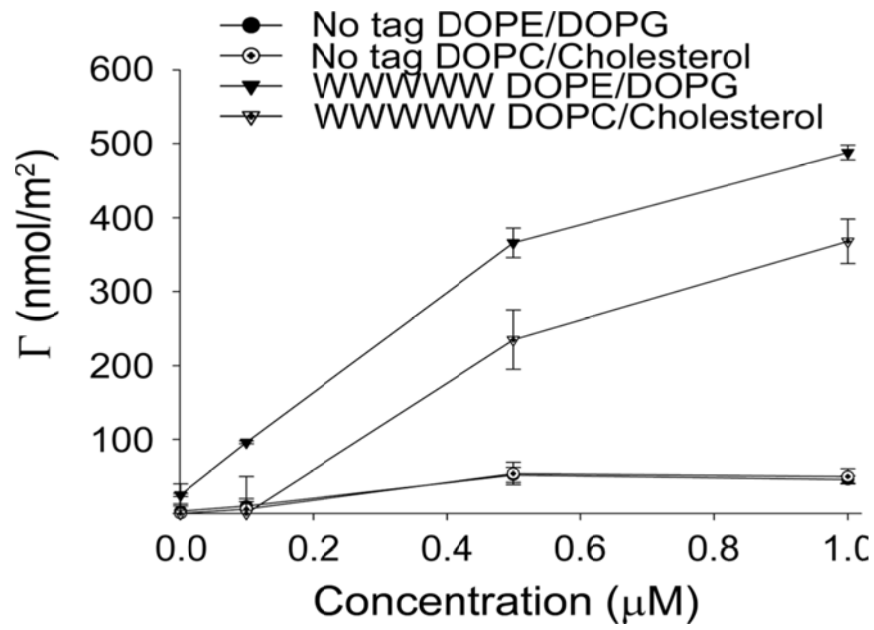

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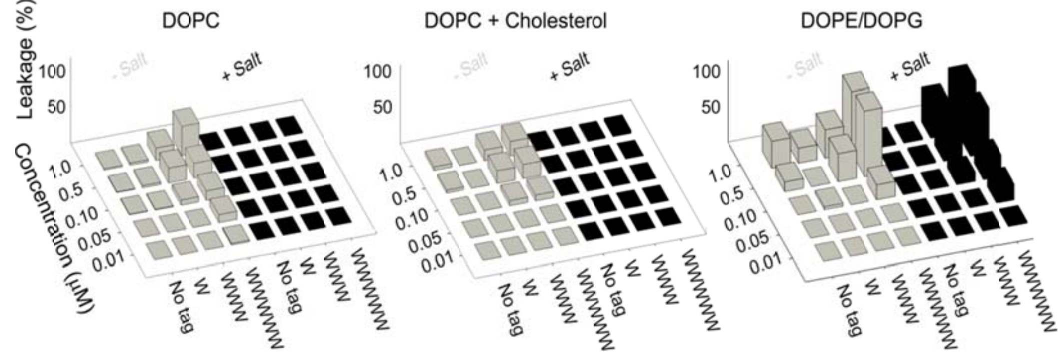

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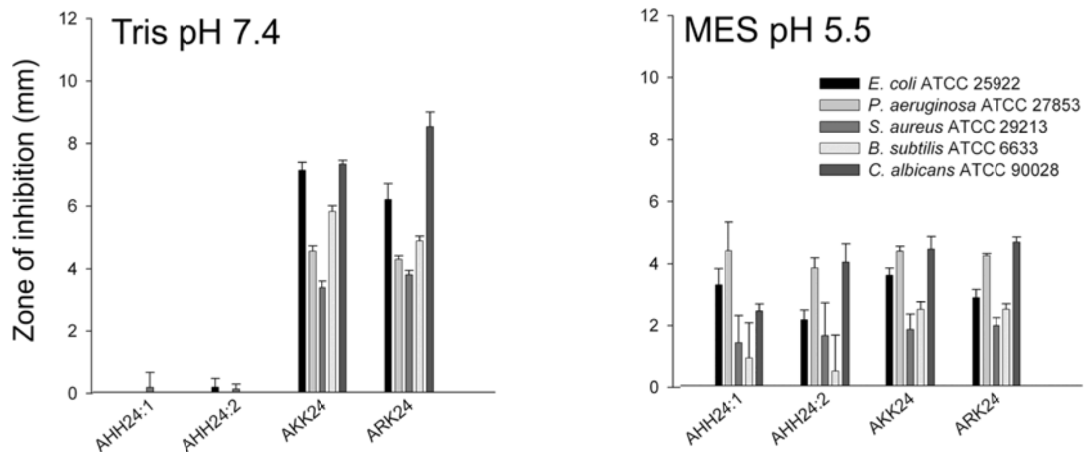

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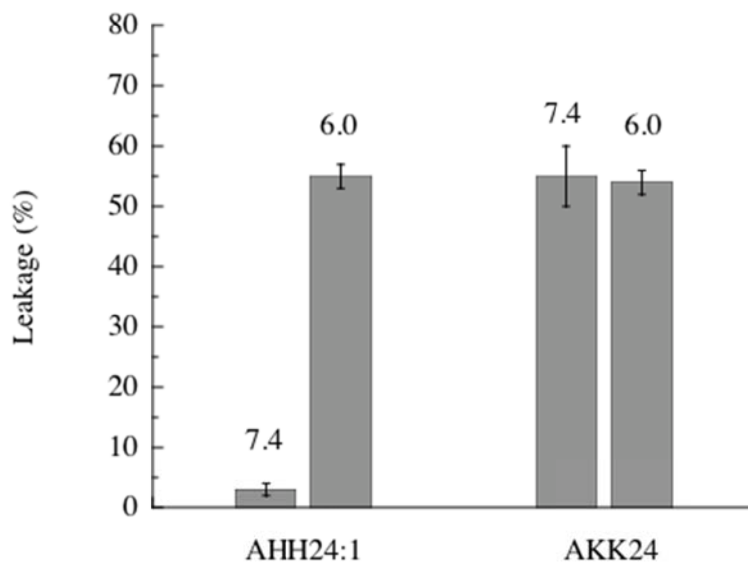

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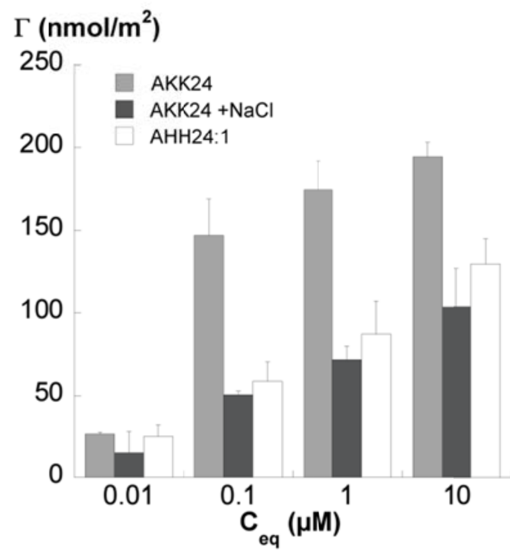

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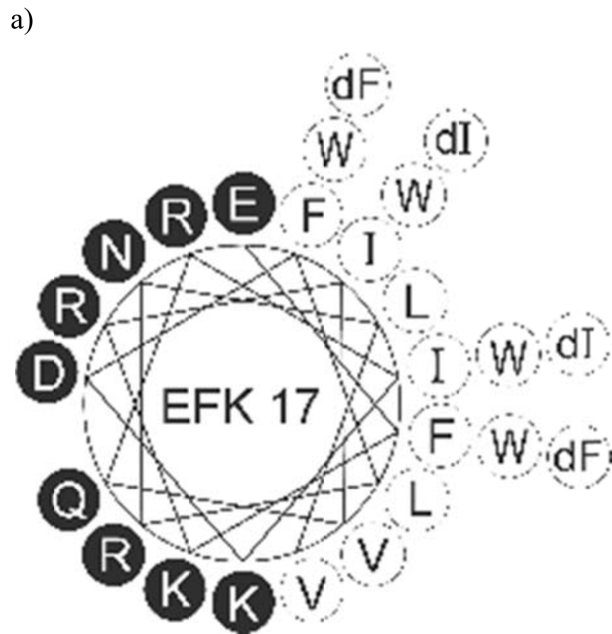

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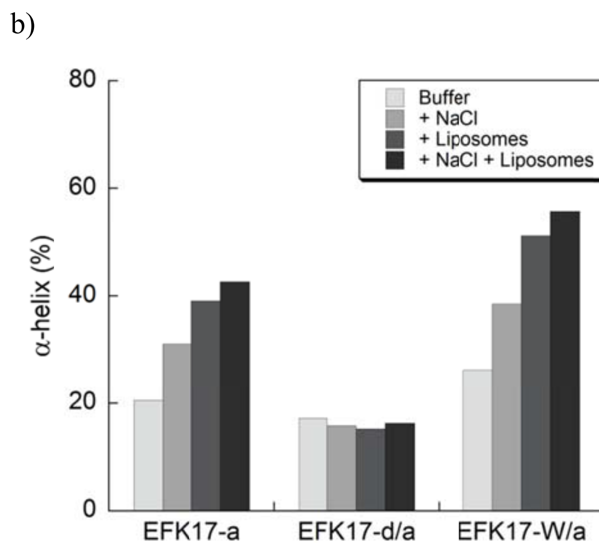

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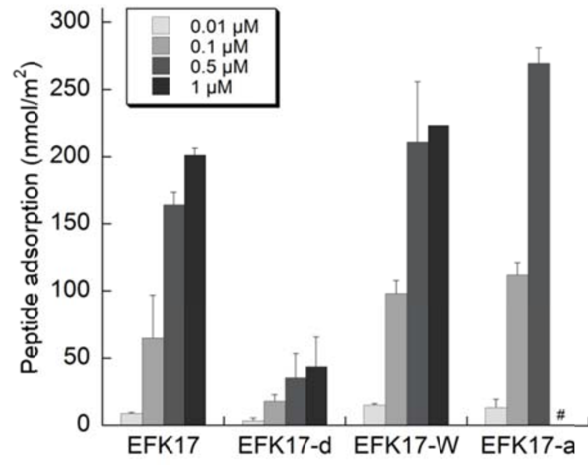

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