spectroscopy on lipid bilayer stacks with TAT 'δ-functionalized' probes to monitor both the TAT position within a single bilayer and the associated force with microsecond resolution. To our knowledge these results present the first direct quantification of the mechanics of TAT penetration and the first demonstration that the different regimes identified in dynamic force spectroscopy correspond to distinct mechanisms. The AFM results show that TAT by itself does indeed alter the membrane structure. Additional results from lysine oligomer probes indicate that TAT's arginine groups are key to these TAT-lipid interactions, since probes functionalized with a lysine oligomer did not induce bilayer thinning. Though TAT strongly interacts with the lipid bilayer, the energy barrier for TAT penetration is actually 38kT higher than for probes functionalized with 11-mercaptoundecanoic acid. These results corroborate many of the conclusions from molecular dynamics simulations on TAT-lipid systems, which indicate that TAT does not penetrate bilayers directly.

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Membrane Fusion is Induced by Antimicrobial and Cell Penetrating Peptides, to an Extent that Correlates with their Conformational Change

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Antimicrobial peptides (AMPs) kill bacteria via membrane permeabilization, whereas cell penetrating peptides (CPPs) can cross cellular membranes without causing damage. Yet, many AMPs and CPPs resemble one another, being short cationic peptides, which tend to be unfolded in solution but assume some kind of amphiphilic structure in the membrane-bound state. Fusogenic peptides (FPs) represent a third functional class, responsible e.g. for viral infection, and they are described as short and hydrophobic sequences with a pronounced conformational plasticity.

Despite their distinctly different biological roles, we have tested the ability of all three classes of membrane-active peptides to trigger membrane fusion. The HIV1 fusion peptide FP23 is used as a reference to compare the fusion activities of several representative AMPs and CPPs with different conformational preferences and compositions. A fluorescence dequenching assay was used to monitor lipid mixing, and dynamic light scattering revealed the size-increase of the fused vesicles. Several AMPs and CPPs were thus found to be fusogenic to an even higher degree than FP23, which had not been expected. Some insight into the reason for this remarkable activity was obtained by monitoring the secondary structure of the peptides in aqueous buffer before, and in the membrane-bound state after fusion. We found a correlation between the extent of fusion and the extent of lipid-induced folding, suggesting that the energy released in the conformational change is responsible for perturbing the lipid packing in the bilayer and thereby triggering fusion.

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Lipid Clustering by Three Homologous Arginine-Rich Antimicrobial Peptides is Insensitive to Amino Acid Arrangement

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Membrane and antimicrobial properties of three short Arg-rich peptides containing the same amino acid composition but different sequences were determined in this study. These peptides, PFWRIRIRR-amide (PR-9), RRPFWIIRR-amide (RR-9) and PRFRWRIRI-amide (PI-9), all exhibit the ability to induce segregation of the anionic lipids from the zwitterionic lipids, as shown by changes in the phase transition properties of lipid mixtures detected by differential scanning calorimetry and also by freeze fracture electron microscopy. The Minimal Inhibitory Concentration (MIC) of these three peptides against several strains of Gram positive bacteria correlated well with the lipid composition of the bacterial membrane. The lower activity of these three peptides against Gram negative bacteria, particularly PI-9, could be explained by the interactions of these peptides with LPS as shown by isothermal titration calorimetry. The promotion of lipid domains by PR-9 as well as by a cathelicidin fragment, KR-12 that had previously been shown to induce lipid phase segregation, was directly visualized using freeze fracture electron microscopy. This work shows the insensitivity of phase segregation to the specific arrangement of the cationic charges in the sequence of these small cationic peptides as well as being independent of their tendency to form different secondary structures.

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N-Acylation of Antimicrobial Peptides Causes Different Mode of Cell Membrane Damage

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Lipopeptides such as polymyxins, octapeptins or daptomycin often show an increased activity against bacteria as compared to their non-acylated analogue. Thus, we have studied N-acylated synthetic peptides derived from a fragment of human lactoferrin (LF-11) to elucidate the interaction of these peptides with Gram-negative and Gram-positive bacteria and membrane mimetic systems using various biophysical and biological methods.

Calorimetric studies on liposomes composed of phosphatidylglycerol revealed that the parent peptide induced a phase separation into peptide-enriched and -poor domains, which however consist of a similar domain size as calculated by the cooperative units. In contrast, at the same lipid-to-peptide molar ratio (25:1) the N-acylated derivatives strongly broadened the phase transition range and lowered markedly the main transition temperature. This is indicative for rather small and inhomogeneous domains, which will result in large line defects increasing membrane permeability as observed in intact bacteria. Membrane destabilization of E. coli and S. aureus induced by the peptides was monitored by using the membrane-potential-sensitive dye DiIC1 and the extent of membrane damage caused by the peptides by the cationic dye SYTOX green, which cannot enter intact cells unless its membrane is disrupted by external compounds. In both assays the N-acylated peptides showed a dramatic increase of fluorescence indicating massive membrane damage. This is supported by electron micrographs, which clearly showed a loss of cytoplasmic content and membrane rupture in the presence of the N-acylated peptide. Nevertheless, the extent of cell membrane rupture does not necessarily strongly correlate with the MIC-value of the peptides emphasizing the different mode of interaction of (non)-acylated peptides, which in part may be related to different degree of interaction with cell membrane/wall components such as lipopolysaccharides and lipoteichoic acid.

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Protegrin-1 Orientation in Membrane Bilayers: Insights from Potential of Mean Force Calculations as A Function of Its Tilt and Rotation Angles

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Protegrin-1 (PG-1) belongs to the family of antimicrobial peptides. It interacts specifically with the membrane of a pathogen and kills the pathogen by releasing its cellular contents. To fully understand the energetics governing the orientation of PG-1 in different membrane environments and its effects on the physicochemical properties of the peptide, we have calculated the potentials of mean force (PMF) of PG-1 as a function of its tilt angle in explicit membrane bilayers composed of either DLPC (1,2-dilauroylphosphatidylcholine) or POPC (1-palmitoyl-2-oleoylphosphatidylcholine) lipid molecules. The resulting PMFs in explicit lipid bilayers were then used to search for the optimal hydrophobic thickness of the implicit membrane, in which a two-dimensional PMF in the tilt and rotation space was calculated. The calculated PMFs in explicit membrane systems clearly reveal that the energetically favorable tilt angle is affected by both the membrane hydrophobic thickness and the PG-1 rotation angle. Local thinning of the membrane around PG-1 is observed upon PG-1 tilting. The thinning effect is caused by different arginines in regard to the rotation orientation of the peptide. The two-dimensional PMF calculated in implicit membrane at specified rotation angles is in good accordance with those from the explicit membrane simulations. The ensemble-averaged Val16 ^{15}N and ^{13}CO chemical shifts calculated from the two-dimensional free energy distribution agree fairly well with the experimental values, suggesting the accuracy and reliability of the application of PMFs to understand important physicochemical properties of membrane peptides/proteins.