1128-Plat
The First Millisecond of the Myosin Working Stroke Under Constant Load
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Myosin II is the motor protein that drives muscle contraction through cyclical interactions with an actin filament. The working stroke produced by a single myosin head has been previously measured in isolated myosin molecules, but the effects of the high loads acting on the myosin molecule during muscle contraction could not be investigated. In fact, current single molecule techniques apply force with a delay of few milliseconds after actin-myosin binding, when the working stroke of skeletal muscle myosin has already been completed.
Here, we developed a novel single molecule technique in which the delay between myosin binding and force application is abolished. This method is capable of resolving the development of the myosin working stroke under different loads with a very high time resolution and detecting events as short as 100 μs. We found that under loads in the range 1 to 10 pN myosin can follow two distinct pathways in its interaction with actin. In the first pathway myosin detaches from actin before producing any movement (weak binding state); these events are very fast (240 ± 23 μs), their duration does not depend on ATP concentration, and is not significantly affected by force. In the second pathway myosin steps and remains bound to actin for a longer time (strong binding state). At low forces ($|F| < 2$ pN) the lifetime of this second population of events linearly decreases with ATP concentration in the range 5-50 μM. At higher forces this relation becomes non-linear due to premature unbinding of myosin from actin.
The working stroke is produced in two steps and its mean amplitude is found to be smaller at increasing loads and vanishes at the isometric force (5.7 ± 0.6 pN).

Platform U: Membrane Active Peptides

1129-Plat
Effect of Molecular Organization in Micelles and Bilayers on Binding and Conformation of Biologically Active Peptides
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Amphiphiles form different types of aggregates, such as micelles and bilayers, depending on their shape and hydrophilic-hydrophobic balance. While bilayers form vesicles containing an inner aqueous compartment, micelles are smaller, approximately spherically-shaped, and have no internal aqueous compartment. Thus, molecular packing and mobility vary in these aggregates, and EPR spectra of spin probes can be used to examine these properties. EPR spectra evince tighter molecular packing and slower rate of motion in bilayers than in micelles. Such differences affect binding of peptides, both qualitatively and quantitatively. Fluorescence, CD, and EPR were employed to investigate interactions of micelles and vesicles with antimicrobial peptides, as well as fragments of GPCR and cytolytic toxins. EPR was also used for peptide analogues containing the paramagnetic amino acid TOAC. Two-component spectra indicated slow exchange between bound peptide and peptide tumbling fast in aqueous solution, allowing the calculation of binding constants. Peptide-membrane interaction was also monitored by changes in peptide fluorescence intensity and emission wavelengths, as well as accessibility to a water soluble quencher. CD spectra showed that upon binding the peptides acquired secondary structure due to formation of intramolecular hydrogen bonds, favored by the decreased polarity at the lipid interface. While in most cases, bilayer binding was only observed when electrostatic interactions occurred between positively charged peptides and negatively charged phospholipids, electrostatic effects played a less important role in peptide-micelle interaction. These differences were ascribed to differences in molecular packing and curvature in both types of aggregates. The positive curvature of micelles is proposed to mimic the lipid organization of toroidal pores. Thus, the conformational behavior in the presence of micelles would correspond to that of peptides forming toroidal pores in bilayer membranes.
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1130-Plat
Cholesterol Effect on The Lipid Bilayer Perturbation Induced by Peptides Derived from the Membrane-Proximal External Region of HIV-1 gp41
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The conserved, membrane-proximal external region (MPER) of the human immunodeficiency virus type-1 envelope glycoprotein 41 subunit is required for fusogenic activity. It has been proposed that MPER functions by disrupting the cholesterol-enriched virion membrane. We have compared the effects of cholesterol on the membrane perturbations induced by N-preTM and PreTM-C, two peptides derived from MPER sequences showing tendency to associate with the bilayer interface or to transfer into the hydrocarbon-core, respectively. Capacities of N-preTM and PreTM-C for associating with lipid vesicles were comparable. However, supporting the existence of different membrane-bound structures, N-preTM established unstable pores that induced permeabilization following a graded mechanism, whereas PreTM-C pores were stable and permeabilized LUVs and GUVs following an all-or-none mechanism. Cholesterol did not alter these permeabilization mechanisms, but affected differently the lytic capacities of the peptides. N-preTM partitioning and induced leakage decreased as the bilayer area compressibility modulus (KA) increased. In contrast, cholesterol highly stimulated PreTM-C-induced leakage under conditions that did not affect partitioning. Finally, fluid phase co-existence stimulated leakage induced by both peptides, which were confined within liquid disordered domains. These results support specific roles for cholesterol in modulating MPER membrane-disrupting effects that are not dependent on raft formation.

1131-Plat
Dependence of Amyloid-β Oligomer (AβO) Interaction with Membranes on Preparation Method
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AβOs reduce the resistance of lipid membranes to ion transfer in a dose-dependent fashion.[1] Combined conductance and structural studies suggest that AβOs at micromolar total peptide concentrations increase the dielectric constant in membrane cores by forming inhomogeneous patches within the membrane,[2] but some issues remain unresolved.[3] Here we compare the conductance increases induced by AβOs in membranes, both tethered and free-standing, for particles prepared by solubilization in hexafluoroisopropanol (HFIP) or NaOH. AβO aggregation and time course, and their association with membranes, are also characterized by their conformation-sensitive reaction with antibodies, dynamic light scattering, and neutron reflectometry. ^{19}F-NMR is used to quantify HFIP content in buffer and detect residual HFIP in AβO preparations. While prolonged evaporation from buffer reduced the HFIP concentration below the NMR detection limit we find that similarly treated AβO samples retain HFIP firmly bound to the peptide at a level of ~ 1HFIP per 5 amyloid peptides. While this amount is probably too low to account for the conductance effects of HFIP-prepared AβOs, we also observe that NaOH-prepared AβOs do indeed induce smaller conductance increases at the same concentrations. This study addresses the differences between HFIP-prepared and NaOH-prepared AβOs and how these may contribute to differences in their conductivity effects on membranes.
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1132-Plat
AFM Force Spectroscopy on TAT Membrane Penetration
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We present a study of the interactions between cell-penetrating peptides (CPPs) and lipid stacks using Atomic Force Microscopy (AFM). Understanding how CPPs can pass through cell membranes is critical for designing optimal drug delivery agents. While CPPs like HIV-TAT, a positively charged 9-mer with six arginine groups, have been widely studied, their precise penetration mechanisms are still not well understood. New experimental methods are needed to characterize CPP behavior and determine whether TAT can penetrate bilayers directly. Direct measurement of TAT-lipid mechanics during the actual translocation event is an ideal method to elucidate the interaction forces, mechanisms and timescales of membrane penetration. We used AFM force

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.

1133-Plat

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.

1134-Plat

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.

1135-Plat

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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1136-Plat

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.