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