measurements have been carried out by the injecting RDH-11, N-del RDH-11 and the synthetic N-terminal peptide (NTP) into the subphase of a phospholipid monolayer at the air-water interface. Their kinetics of monolayer binding, monitored by surface pressure measurements, increases as follows : NTP > RDH-11 > N-del RDH-11. Moreover, measurements by polarization-modulated infrared reflection absorption spectroscopy have allowed to confirm the alpha helical structure of the NTP and to determine its orientation as well as to compare the structure and orientation of RDH-11 and N-del RDH-11. For example, compared to the pure protein, N-del RDH-11 undergoes a conformational change upon monolayer binding.

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Calcium Independent Substrate and Product Diffusion Process of Secretary Phospholipase A2 from Taiwan Cobra

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Understanding how membrane lipids can be delivered to the active site distant from the interfacial binding surface of the enzyme and how the products got released from the active site are important to depict the interfacial enzyme reaction mechanism. Based on the crystal structure of the trimeric complex structure of the cobra phospholipase A2 (PLA2) from Naja atra with the enyzymatic substrate of diacylheptanoyl phosphatidylcholine (PC) and products of lyso PC and fatty acids (minic by SDS), we suggest that promiscuous bindings of phospholipids to the interfacial enzyme may boost the lipid desorption process via a cooperative hydrophobic interaction among the hydrocarbon chains of phospholipids and that with the interfacial surface of the enzyme. We also show that phospholipids in membranes surface with high curvature can promote the diffusion of the lipid into the substrate binding hydrophobic channel of cobra PLA2 in a calcium independent manner. The PC substrate binding site within the channel without calcium is distinct from that in the presence of calcium as one compares its binding position with that of transition binding intermediates. Interestingly, calcium appears to destabilize the binding of both substrate and product binding at the hydrophobic channel even though it is required for the enzymatic catalysis. Our results suggest that the calcium independent lipid diffusion process play an important role in the interfacial binding activation of secretary PLA2 and shed new light for the future depiction of the energy landscape.

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A New Conformation in SERCA and PMCA Ca^{2+} Pumps Revealed by a Photoactivatable Phospholipidic Probe

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The purpose of this work was to obtain structural information about conformational changes in PMCA membrane regions and their interaction with surrounding lipids. To this end, we have quantified labeling of the sarcoplasmic reticulum Ca^{2+} pump (SERCA) and the plasma membrane Ca^{2+} pump (PMCA) with the photoactivatable phosphatidylcholine analog [^{125}I]TID-PC/16, under different conditions. This probe has been used previously to analyze lipid-protein interfaces. We determined that: (1) Incorporation of the photoactivatable reagent to SERCA decreases 25% when labeling is performed in the presence of Ca^{2+} as opposed to EGTA (2) The decrease in labeling matches qualitatively with the decrease in transmembrane surface exposed to the solvent calculated by the Lee-Richards method, when comparing the known SERCA structures 2ear (E_2) (*pdb. file*) and 1su4 (E1Ca) (*pdb. file*). (3) Labeling of PMCA incubated with Ca^{2+} and calmodulin decreases by almost the same amount as compared to EGTA. However incubation with Ca^{2+} alone (no calmodulin) increases labeling by 55%. This suggests that the conformation in which the enzyme is fully active (Ca^{2+} for SERCA and Ca^{2+}-CaM for PMCA) exhibits a more compact transmembrane arrangement in both proteins. Addition of C28, a peptide containing the calmodulin binding region of PMCA, to SERCA in the presence of Ca^{2+} increases [^{125}I]TID-PC/16 incorporation, confirming the suggestion made above. The results indicate that there is an autoinhibited conformation in these P-type ATPases that affects not only the cytoplasmic regions but also the transmembrane segments.

Muscle: Fiber & Molecular Mechanics & Structure II

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***In Vitro* Study of Mechanical and Kinetic Properties of Myosin II from Frog Skeletal Muscle**

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We provide for the first time the protocol for efficient extraction and conservation of myosin II from frog skeletal muscle, a methodological achievement that makes it possible to apply single molecule techniques to the molecular motor that has been best characterized for its mechanical, structural and energetic characteristics in single muscle cells, where it works as an ensemble in each half-sarcomere. With the *in vitro* motility assay, we estimate the sliding velocity of actin on frog myosin II (V_F) and its modulation by temperature (range 4-30 °C) and substrate concentration. V_F is 8.88 ± 0.511 μm/s at 30.6 °C and decreases down to 1.6 ± 0.23 μm/s at 4.5 °C. The *in vitro* mechanical and kinetic parameters are integrated with the *in situ* mechanical and kinetic parameters of frog muscle myosin working in array in each half-sarcomere. By comparing V_F with the shortening velocity determined in intact frog muscle fibres under different loads and their dependence on temperature (Piazzesi et al., *J. Physol.*, 549:93, 2003), we find that V_F is 40-50% less than the *in situ* unloaded shortening velocity (V_0) at the same temperature and we determine the load that explains the reduced value of V_F. With the integrated approach we can define fundamental kinetic steps of the acto-myosin ATPase cycle *in situ* and their relation with mechanical steps. In particular we clarify the relation between the rate of ADP release and the rate of detachment of myosin from actin and their temperature dependence. Supported by NIH (Grant no. 5R01AR49033) and MiUR Italy.

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Construction Of Myosin Model Explaining Difference Between Experimental Results Observed With Scanning Probe And Optical Tweezers

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Single molecule measurement (SMM) techniques have been applied to myosin. Then, SMMs' results show that, during single ATP hydrolysis cycle, myosins II & V repeat several cycles of association with- and dissociation from an actin filament to generate sliding motion, suggesting that myosin can convert ATP energy by multi-step processes (MSPs). This MSPs cannot be explained by conventional ''lever-arm model'', then ''Biased Brownian motion (BBM) model'' has been proposed for a mechanism of myosin. However, the MSPs have been observed only by SMM with scanning probe (SP), and not observed with optical tweezers (OT) widely used for SMM. Because MSPs have been observed clearly with myosin II & V, it is strongly suggested that BBM is movement mechanism of myosin. Then, why have MSPs not been observed with optical tweezers? In order to answer this question, here, we construct model including characteristics of SP & OT, and simulate movement of myosin attached to measurement probes (SP or OT). Taking into account the effects of measurement probes, we construct 2-demensional potential along an actin filament, and simulate movement of myosin on the 2D potential by Monte Carlo method. For simulation, spring constants of probes parallel and perpendicular to an actin filament are set according to characteristics of each probe. As a result, sliding velocity with SP (~0.5 um/s) becomes slower than that with OT (~3 um/s), then MSPs are clearly observed with SP. This result explains well the experimental results with SP and OT.