2P132 ミオシン V における ADP 解離の負荷方向角度依存性
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Angular dependence of ADP dissociation kinetics in myosin V under directional loading
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Individual molecules of myosin V can travel along an actin filament using two identical heads. For such motion, there should exist a property, which is different between the leading and the trailing heads during the double-headed binding to actin. It is thought that ADP dissociation from the trailing head is facilitated compared to the leading head, and this asymmetrical property may be derived from the internal strain existing between the leading and trailing heads. Actually, at the last year BSJ annual meeting we showed that the ADP affinity of the single-headed myosin V is different under the forward and the backward load; in addition, this dependence of the ADP affinity on loading direction became more pronounced when load was applied by some angle to the long axis of the actin filament. However, these ADP affinities contain both the ADP dissociation and binding kinetics, so it is important to evaluate the load dependence of the ADP dissociation rate only, to reveal the myosin's V walking mechanism more precisely. Therefore, we established the model which reproduces those experimental results well, and this model analysis revealed the extent of the effect of load on the ADP dissociation rate at each angle of loading direction. We will discuss at this meeting that such load dependence of the ADP dissociation kinetics is a basis for the effective processive movement of myosin V.

2P134 高速 AFM によるアクトミオシン V の動態観察
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Structural dynamics of acto-myosin V revealed by high-speed AFM
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Myosin V is a two-headed molecular motor that delivers intracellular cargos over a long distance by moving processively along F-actin. Although numerous studies support the hand-over-hand model, its detailed behavior at nano-meter resolution is not elucidated yet. Here, we tried to visualize it by using the most recent version of our high-speed AFM. As the substratum used for AFM imaging, we used supported planar lipid bilayers that resist protein adsorption. First, we found characteristic binding manners (i.e., single-head binding or double-heads binding) of actin-myosin V that depended on the stationary chemical states (nucleotide-free, ADP and AMPPNP). In ATP, both the myosin V heads bound to the same F-actin at sites spaced ~ 36 nm apart. Then, the leading neck region bent just before the trailing head detached from the F-actin. This bending seems to result from the rotation of the converter domain in the leading head which is coupled with transition from the weakly bound state to the strongly bound state. The detached trailing head rotated around the junction between the two lever arms, then landed on a forward actin site ~ 72 nm apart from the previously bound site, and thus became the new leading head. Just after the landing, the new leading head sometimes showed stepping motions biased toward the plus end of the F-actin. The step size was ~ 5.5 nm. These AFM movies directly showed a series of structural changes in myosin V during the hand-over-hand movement and revealed the mechanism that makes this movement possible.

2P133 頭部を基板に固定したミオシン V-S1 でのアクチン滑り運動
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Actin gliding over myosin V S1 tethered on a glass surface at the head domain
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The movement of myosin V has been supposed to be driven by swinging lever arms. In the swinging lever arm model, the head domain converts its conformation by the ATP hydrolysis to swing the lever arm. In addition, myosin V motor activity is regulated by Ca²⁺. In high Ca²⁺, one calmodulin dissociates from each IQ2 on the neck regions, resulting in the motor activity loss. Here, we sought to find a new aspect of the motor mechanism. Chick brain myosin V (BMV) was labeled with biotin-PEG-maleimide (M.W. 3400) at its heads and digested at its neck (at the IQ2-3 junction) by proteinase K. This sample (BMV-S1) was tethered onto glass surface via streptavidin and then actin gliding assays were performed using TIRF microscopy. If BMV-S1 follows only the swinging lever arm model, actin filaments should not move in this assay because the movement of the converter domain, which exists in the head in close proximity to the neck, cannot be transferred to the head portion anchored onto the surface. However, actin filament gliding was observed, although it occurred at a low speed (approx. 10 nm/sec). In addition, this gliding movement was observed not only in EGTA, but also in the presence of high Ca²⁺. These results do not seem to reconcile with the simple swinging lever arm model and suggest two possibilities: (1) an additional motor mechanism exists, or (2) the head portion also swings, which is not regulated by Ca²⁺.

2P135 逆方向に動くミオシン V・ミオシン VI は、連続的な運動において、同様に両足を協調する
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Two heads of the oppositely directed myosins V and VI are similarly coordinated during processive stepping
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Individual molecules of unconventional myosins V and VI travel long distances toward opposite ends of single actin filaments, taking multiple ~36 nm steps in a "hand-over-hand" manner before dissociating. Models of how they "walk" implicate the state of a double-headed binding to actin, supposedly in the ADP state. ADP release from the trailing head allows ATP to bind, leading to dissociation of this head from actin and stepping forward. For efficient directional processive movement of a dimeric myosin via a "hand-over-hand" mechanism, the two heads must communicate with each other, resulting in the easier dissociation of ADP from the trailing head than from the leading head. Here we present direct experimental evidence that external loads applied to individual actomyosin V or VI bonds affect ADP affinity in an asymmetric manner, such that ADP binds weakly under loads acting in the direction of motility and strongly under loads opposing motility. To more thoroughly evaluate the mechanism of load-induced modulation of ADP dissociation kinetics, we performed the model analysis, based on the experimental data, to reveal the effect of load on both ADP dissociation and binding rates. These results help establish general principles governing the efficient unidirectional processive movement of dimeric ATP-driven molecular motors and provide strong support to models in which intramolecular strain generated during double-headed binding to actin coordinates the catalytic cycles of the two heads.