shown that myosin X adopts a left-handed helical path along these cytoskeletal structures, consistent with its step size. The radius of the helical path followed by the quantum dot increases between labeling sites on the CaMs and the C-terminus and between single filaments and bundles. The radii suggest flexibility in the tail. These features of the motility, in conjunction with membrane and microtubule binding domains, enable myosin X to operate on varied actin structures in multiple cellular functions. Supported by NSF NSEC grant DMR04-25780 and NIH grant GM086532.

752-Pos

Influence of Actin Mutant to Procesive and Non-Processive Myosin Motility

Tomotaka Komori1, Hiroaki Takagi2, Masatoshi Nishikawa3, Atsuko H. Iwane1, Toshiro Yanagida1
1Osaka University, Suita Osaka, Japan, 2University of Technology, Gothenburg, Sweden, 3Osaka University, Suita Osaka, Japan.

The myosin family is an ATP driven molecular motor that interacts with an actin filament via ionic bonds. In particular, there are eight specific, negative charged amino acids in actin that match with eight positively charged amino acids in the myosin II region. However, how these ionic bonds relate to the step size remains to be elucidated. Here, we constructed several actin mutants in which the number of negatively charged amino acids were decreased (0 to 6) or increased (10 to 12). To clarify these points, we performed actin gel filtration assays using myosin-II and -V, separately. The actin gel filtration on myosin-V was accelerated with a decrease in negative actin charge, although we did not see processive movement in single molecule imaging measurements. On the other hand, actin gel filtration on myosin-II decreased regardless of increasing or decreasing the number of negative charges in actin relative to WT. These results indicate that the number of negative charges in WT actin is well tuned for processive and non-processive myosin motility. At present, we are planning to perform additional analysis including biochemical assays and single molecule measurements to further test this hypothesis. Additionally, we are investigating differences in the stepping mechanism between processive and non-processive myosins with respect to the actomyosin interaction.

753-Pos

Differential Effects of Alpha Vs Beta Myosin Heavy Chain on the Kinetics and Mechanics of Familial Hypertrophic Cardiomyopathy

Michael J. Greenberg1, James D. Watt1, Katarzyna Kazmierczak2
1University of Pavia, Dept Physiology, Pavia, Italy, 2University of Kent, Dept of Biosciences, Canterbury, United Kingdom.

Cardiac muscle myosin is comprised of two heavy chains (MHCs), two essential light chains, and two regulatory light chains (RLCs). The MHC contains both the ATPase and actin binding domains. It has been shown that the contractile properties of myosin can be tuned by the MHC isoform and that the MHC isoform distribution in the human heart changes during heart failure from predominantly beta isoform to alpha. One cause of heart failure is familial hypertrophic cardiomyopathy (FHC) which is triggered by mutation of sarcomeric proteins including the RLC. Although the RLC is spatially separated from the myosin active site, it appears to have a role in tuning myosin kinetics. In order to examine how two RLC mutations implicated in FHC, N47K and R55Q, affect the kinetic and mechanical properties of beta isoform myosin, we exchanged porcine cardiac RLC with recombinant mutant RLC. We examined the contractile properties of these mutants using the in vitro motility assay and compared these results to our earlier results with mutant RLCs on the alpha-MHC background. Regardless of MHC isoform, the mutations cause reductions in force and power output. However, on the alpha MHC backbone, R55Q shows differences in calcium handling and an elevated ATPase rate which is not seen on the beta backbone. Also, both mutants show increases in duty cycle on the alpha MHC but not the beta. These data suggest that small changes in the myosin structure, far from the active site, can disrupt the contractile properties of the motor depending on the MHC isoform.