Shape changes in myosin molecules during ATPase and regulation of activity

Peter Knight, Stan Burgess, Matt Walker, Neil Billington, Hyun Suk Jung, Olusola Oke, Nathan Swailes, Kavitha Thirumurugan and John Trinick

Myosin V

The vast majority of work on myosin has studied the class II molecule which is the class found in muscle. However recent years have seen the identification of 17 other myosin classes, from which most human cells express several. In a paper published in Nature in 2000 we showed for the first time electron micrographs of class V myosin with both its heads simultaneously attached to actin. We suggested that the leading head in such molecules was at the start of its power stroke, and that these were the first images of any myosin at the start of its stroke. Determination of this conformation has been the single greatest impediment to understanding the molecular mechanism of force production by myosins.

Following on from this work, we have now studied the conformations of the heads of myosin V detached from actin in the presence of ATP and in the absence of nucleotide. Crystal structures of myosin II heads in several long-lived nucleotide analogue states have been solved. However, crystallography is incompatible with protein molecules executing gross conformational changes during their catalytic cycle; moreover, there has been controversy as to which states of the ATPase cycle the long-lived analogues represent. Our new data reveal that, in ATP, about half the heads are in a strongly bent (~90°) conformation that is similar to crystal structures of myosin II heads containing ADP and AlF_4 or ADP and vanadate which have been proposed to represent the conformation at the start of the power stroke. This conformation is also similar in several respects to the lead heads in molecules attached to actin by both heads. However, the lead head is even more strongly bent, probably by being restrained through the trail head. This distortion of the molecule in the attached state is likely to be important in the processive stepping mechanism of myosin V along actin filaments.

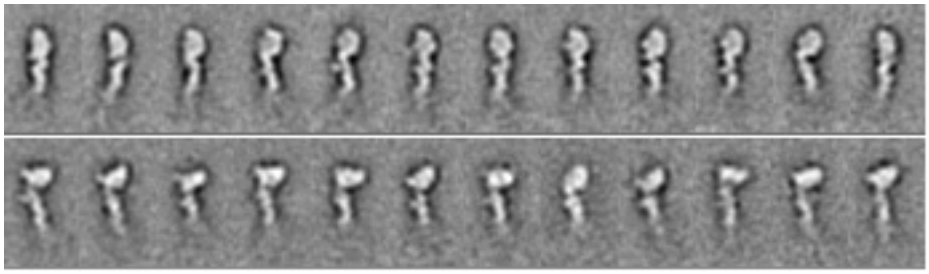

Fig. 1 Myosin V heads in the absence or presence of ATP (upper and lower rows respectively). Images obtained by alignment, classification and averaging of the head regions of negatively stained molecules. Note the large change induced by ATP in the angle between the ~8 nm motor domain and the 'lever arm'.

We have also obtained micrographs of a mutant myosin V that has two alanine residues inserted near the mid-point of the α-helical 'lever arm' part of the molecule. This is expected to result in a 205° rotation in the lever arm, however these molecules are still able to attach to actin with both heads simultaneously, possibly as a result of each head having the freedom to rotate about its long axis.

Smooth muscle myosin II

Smooth and non-muscle myosin II can form an inactive 'hairpin' conformation where the tail folds back on itself to interact with the heads, and the heads bend down towards the tail. We have determined the structure of this conformation by electron microscopy and image processing of both the whole molecule and molecules with a truncated tail. These show the two heads differ in shape and interact asymmetrically, and that the folded tail interacts primarily with one head. The structure and arrangement of the heads is strikingly similar to a structure recently published derived from electron microscopy of 2-D crystals of a short-tailed mutant myosin, but the tail takes a substantially different path.

Collaborators

James Sellers, NIH; Howard White, Eastern Virginia Medical School; Joseph Chalovich, Charlottesville.

Publications

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