A stable single α-helix can act as a lever in myosin

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Introduction

The α-helix (first identified by W. T. Astbury) is a secondary structure commonly found in proteins, either as part of globular proteins or paired with one or more adjacent α-helices in coiled coils, such as in the 'leucine zipper' of the transcriptional regulator GCN4. It is generally thought that single α-helices are inherently unstable in aqueous solution. We recently discovered that secondary structure prediction software incorrectly assigns as coiled coils, sequences that lack the hydrophobic seam that holds the chains together. Several such sequences occur in members of the myosin family of motor proteins. We found that one such sequence from myosin 10 instead forms a monomeric α-helix that is more stable against denaturation by heat or salt than the α-helices of coiled coils. A characteristic of this single α-helix (SAH domain) appears to be large numbers of both acidic and basic amino acid residues that are able to form salt bridges between successive turns of the helix and thus compensate effectively for the tendency of water to disrupt the H-bond network that stabilises the α-helical fold. We have since found that such sequences are widely distributed both across the biome and in proteins of diverse compartments of the cell. However the roles of the SAH domain in proteins remain obscure. In myosins the SAH domain is located adjacent to the lever that amplifies the small, ATP-driven changes in the motor domain into large movements of cargo, and we suggested that it might act as a lever extension. We have recently put this proposal to the test.

Properties of a chimeric myosin

Our test consisted of replacing a large part of the lever of the well-studied myosin 5 with a putative, long SAH domain from a different myosin (MyoM from the slime mould *Dictyostelium discoideum*), and then determining to what extent this chimeric protein behaved like wild type myosin 5 in a battery of test assays.

Myosin 5 is a dimeric myosin in which two heads each consisting of a motor domain and a lever are linked together by a coiled-coil tail (Figure 1). The lever of myosin 5 consists of 6 calmodulin molecules bound close together along an α-helical extension from the motor (which, unlike a SAH domain, collapses if the calmodulins dissociate). The calmodulin-binding sites on this helix are called IQ motifs (because they contain a consensus sequence IQxxxRGxxxR). We deleted 4 of the 6 IQ motifs (creating Myo5-2IQ) and spliced in the putative SAH domain between the IQ motif and the tail to create Myo5-2IQ-SAH (Figure 1).

Electron microscopy of Myo5-2IQ-SAH showed the SAH domain was straight and 17-nm long as predicted from the number of amino acid residues and the rise per residue of an α-helix (0.15 nm), restoring the truncated lever to the length of wild type (Myo5-6IQ). The powerstroke (21.5 nm) produced by single molecules interacting with a single actin filament, as measured in the optical trap, was slightly less than that for Myo5-6IQ but much greater than for Myo5-2IQ, indicating that the SAH domain can indeed extend the mechanical lever action. Myo5-2IQ-SAH moved processively along actin at physiological ATP concentrations with similar step and run lengths to Myo5-6IQ during in-vitro Total Internal Reflection Fluorescence Microscopy assays. In comparison, Myo5-2IQ is not processive under these conditions.

In wild type myosin 5 walking along an actin filament, the trailing head inhibits release of ADP from the leading head (i.e. acts as a gate), and this is presumed to occur by mechanically-preventing the shape change in the leading head that is required for ADP release. For the Myo5-2IQ-SAH chimera, solution biochemical experiments indicated that the

rear head did not mechanically gate the rate of ADP release from the lead head, unlike Myo5-6IQ. These data show that the SAH domain can form part of a functional lever in myosins although its mechanical stiffness might be lower. More generally, we conclude that SAH domains can act as stiff structural extensions in aqueous solution and this structural role may be important in other proteins.

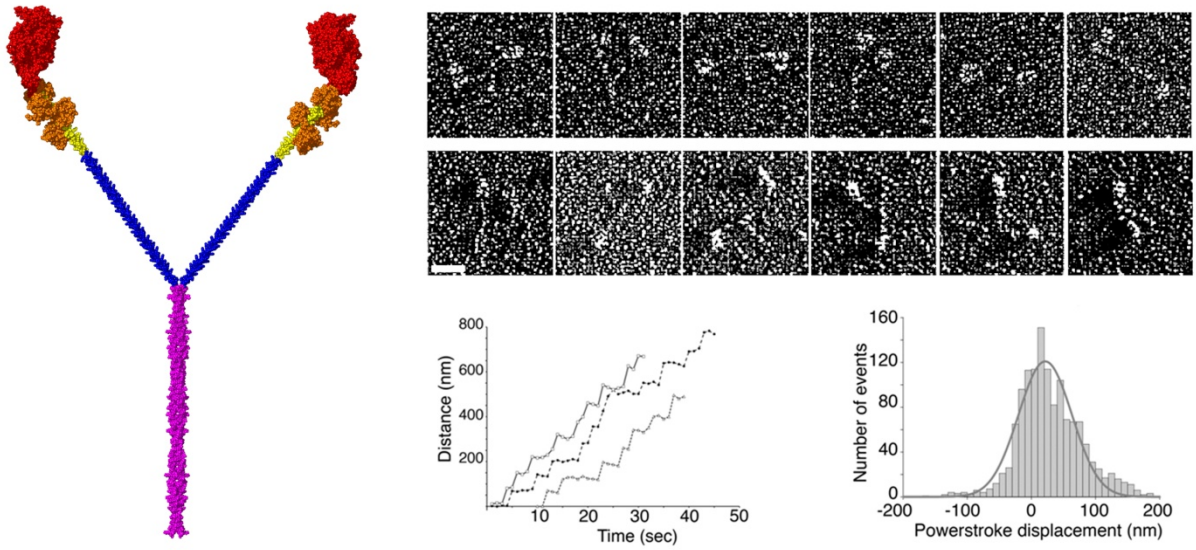

Figure 1: Chimera of myosin 5-2IQ with SAH domain of MyoM. **Left**: atomic model of the chimera; myosin 5 motors red, IQ helix yellow, calmodulins orange, coiled-coil tail mauve; SAH domain blue. **Top right**: EM of single molecules seen by metal shadowing; the thin SAH domains can be seen; scale bar is 25 nm. **Bottom centre**: TIRF assays show strides taken by molecules along actin, that average 70 nm. **Bottom right**: Powerstroke in optical trap averages 22 nm.

Publications

Baboolal, T., Sakamoto, T., Forgacs, E., White, H., Jackson, S., Takagi, Y., Farrow, R., Molloy, J., Knight, P., Sellers, J. and Peckham, M. (2009) The SAH domain extends the functional length of the myosin lever. *Proc Natl Acad Sci USA*, **106**:22193-22198.

Peckham, M. and Knight, P. (2009) When a predicted coiled coil is really a single α -helix, in myosins and other proteins. *Soft Matter*, **5**:2493-2503.

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