Structure of switched-off smooth muscle myosin

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Introduction

The muscles that drive movement in the gut, contraction of the uterus and that maintain tone in the walls of arteries are all smooth muscles. They lack the highly organised structure seen in the striated muscles of the heart and skeleton, but still use a system of myosin and actin filaments to produce contractile force, albeit the myosin filaments have a different architecture. Myosin filaments can be depolymerised by high salt concentrations, and myosin is then seen to comprise two 16 nm heads attached to one end of a ~160nm tail. The heads are the motors of contraction, containing the ATPase and actin-binding sites, while the tail, a canonical α-helical coiled coil, binds to other tails to hold many myosin molecules together and it transmits the forces generated by the heads.

Smooth muscle contraction is regulated by phosphorylation of a regulatory light chain in each head by a specific kinase, which switches on contraction. Relaxation follows dephosphorylation by protein phosphatase. It has been known since the 1980s that *in vitro* the active smooth muscle myosin filaments depolymerise into individual molecules when dephosphorylated. Moreover, unlike myosin in high salt, the tail of these molecules is folded up, roughly into thirds, so that a distal part of the tail is associated with the heads. In this state the ATPase of myosin is essentially zero, and the molecule does not bind to actin. Folded myosin may therefore be able to diffuse or be carried in the cell to allow remodelling of the contractile apparatus in the quiescent muscle. Evidence is accumulating that *in vivo* a significant pool of this monomeric myosin is present, although many myosin filaments persist.

Structure of switched-off myosin

Considering the significance of the folded molecule, surprisingly little detailed work has been done on its structure. We have redressed this by using electron microscopy of single folded molecules from turkey gizzard muscle in negative-stained specimens, followed by single particle image processing (Fig. 1). We have studied both whole myosin and a proteolytic fragment (heavy meromyosin, HMM) that lacks most of the tail, but is still regulated.

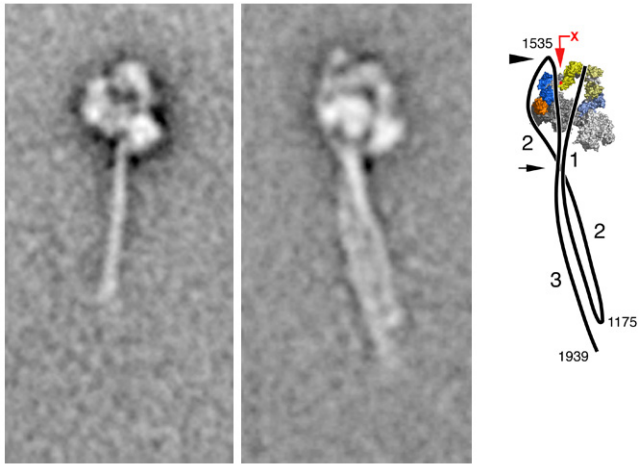

Fig. 1 Conformation of switched off smooth muscle myosin as seen by electron microscopy and image processing. Left panel is the HMM fragment that lacks ~2/3 of the tail; centre panel the intact myosin molecule. The right panel is a diagram of the folded molecule in which the heads derive from an atomic model, with various parts coloured and a black line represents the path of the folded tail. The three segments of the tail are labelled, as are the estimated amino acid sequence positions of the bends. The black arrowhead marks the second bend visible as a pale spot in the image, the arrow highlights where the tail segments are especially close, and the red arrow points at a site on the left head that had previously been shown to be close to the tail.

In both myosin and HMM the two heads are arranged in a very specific and asymmetric way, in contrast to the free movement of the heads around the head-tail junction seen in high salt concentrations. The arrangement is strikingly similar to a structure found in an earlier study when the HMM formed 2-dimensional crystals on a lipid monolayer, and shows that that structure was not an artefact. We have found that the folded molecule is more compact than previously thought. The three segments of the tail are grouped together, and interact in a very specific way with just one of the two heads. We further found that the interaction of the folded tail with the heads stabilised the specific conformation of the heads, and this probably accounts for the very low ATPase of myosin compared to HMM, where these interactions are absent.

Quantifying flexibility of the myosin tail

We have also analysed the structure of the tail. The lengths of the three segments are precise, which implies that the sites of bending in the tail are also precise (Fig. 1). It is not yet clear why the tail bends at these points rather than elsewhere. We have used image processing to examine the variation in the shape of the tail between molecules. For HMM, the single tail curves to a variable degree along its length. On the assumption that this appearance is due to the Brownian forces we have estimated the Young's modulus of the coiled coil tail to be 0.5 GPa. This is close to an earlier value obtained by light scattering from myosin in solution. The folded tail of the intact molecule appears less flexible, as expected if the three segments interact to counter thermal perturbation. This analysis shows that our electron microscopy method can be used to explore the thermally-induced flexibility within macromolecules.

Collaborator

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Publications

Burgess, S. A., Yu, S., Walker, M. L., Hawkins, R. J., Chalovich, J. M. & Knight, P. J. (2007). Structures of smooth muscle myosin and heavy meromyosin in the folded, shutdown state. *J. Mol. Biol*. **372**, 1165-1178.

Funding

This work was funded by BBSRC and NIH.