Molecular basis of myosin assembly: coiled-coil interactions and the role of charge periodicities

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Summary

Complementation of alternating zones of positive and negative charge in the myosin rod enables molecules to interact in a number of ways. This accounts for the complexity of the molecular organisation of thick filaments. However, directed mutagenesis of expressed LMM cDNA indicated that charge zone complementation is not a major driving force in myosin polymerisation. Instead, it probably serves to prevent unfavourable interaction geometries.

Key words: myosin, assembly, structure, mutagenesis, expression.

Introduction

The function of myosin II as a motor protein in muscle and non-muscle cells depends on its ability to polymerize into macromolecular assemblies (thick filaments) that anchor the molecules and enable them to produce force concertedly (see, for example, Squire, 1981). Skeletal muscle thick filaments are stable structures that reflect the rigid architecture of a permanent force-producing machine. In non-muscle cells, however, the organization of myosin is more dynamic, adapting to the needs of cell motility, the maintenance of cell shape, and cell division. Bipolar filamentous molecular arrays similar to native thick filaments can be produced using purified myosin alone. Therefore, although other factors are almost certainly important for the precise arrangement of molecules found in thick filaments *in vivo*, the assembly properties of myosin are largely determined by the interacting surfaces of myosin molecules themselves. Here we combine structural and protein engineering data to explore the molecular basis of the interactions between myosin molecules.

Structure of myosin supramolecular assemblies

Muscle thick filaments are the archetypical myosin assembly. Myosin molecules have rod-like tails and two globular heads and pack into bipolar molecular arrays in which the tails form the thick filament shaft, with the heads arranged on the filament surface in an approximately helical manner. Arthropod thick filaments are 4-stranded helical structures and 3-D reconstructions show a regular arrangement of overlapping myosin heads on their surface (Crowther *et al.* 1985; Stewart *et al.* 1985). Vertebrate thick filaments are 3-stranded and contain 294 myosin molecules. These filaments have a more complicated structure in which the myosin heads are displaced from the positions expected in a perfectly regular helix (Stewart and Kensler, 1986). It is likely that perturbations in the positions of the heads on the filament surface may reflect the fact that the underlying tails are not all arranged equivalently.

Although a good deal is known about the positions of the myosin heads in filaments, there is only scant information about the positions of myosin tails. Transverse sections suggest that there are subunits spaced about 3–4 nm apart, which probably represent molecular dimers (Stewart *et al.* 1981), but it has not been possible to establish the position of individual myosin molecules in filaments. It is important to note that there are a number of key differences between *in vivo* and *in vitro* myosin assemblies and so artificial myosin aggregates may not always reflect the *in vivo* packing precisely. Also, the assembly properties of smooth muscle and non-muscle myosins appear to differ in a number of ways from vertebrate skeletal myosin. In particular, their assembly properties may be modulated *in vivo* by phosphorylation.

Myosin coiled-coil structure

The tails of myosin molecules are 2-stranded alpha-helical coiled-coils. Coiled-coils are a common structural motif in fibrous proteins (e.g. intermediate filament proteins and tropomyosin) and are also present in a range of other proteins where they may form important interaction sites involving such motifs as 'leucine zippers'. In skeletal muscle, the myosin coiled-coil tails interact in the core of thick filaments, with the globular myosin heads arranged in a roughly helical manner on the surface. Coiled-coils are formed by alpha helices winding around one another so that the side chains from one helix fit into the gaps between the side chains of the other. This 'knobs into holes' packing is favoured if the side chains at the interface are hydrophobic. Because the alpha helix has roughly seven residues in two turns, this produces a periodic heptad repeat, *a-b-c-d-e-f-g*, with residues *a* and *d* hydrophobic. Because small changes in the dimensions of the alpha helix produce large variations in the coiled-coil pitch, it is difficult to determine this important parameter from theoretical considerations alone (Fraser and MacRae, 1973).

Direct determination of the coiled-coil pitch was achieved by using electron microscopy and image processing (Quinlan and Stewart, 1987) to solve the structure of crystalline sheets of a proteolytic fragment of myosin rod

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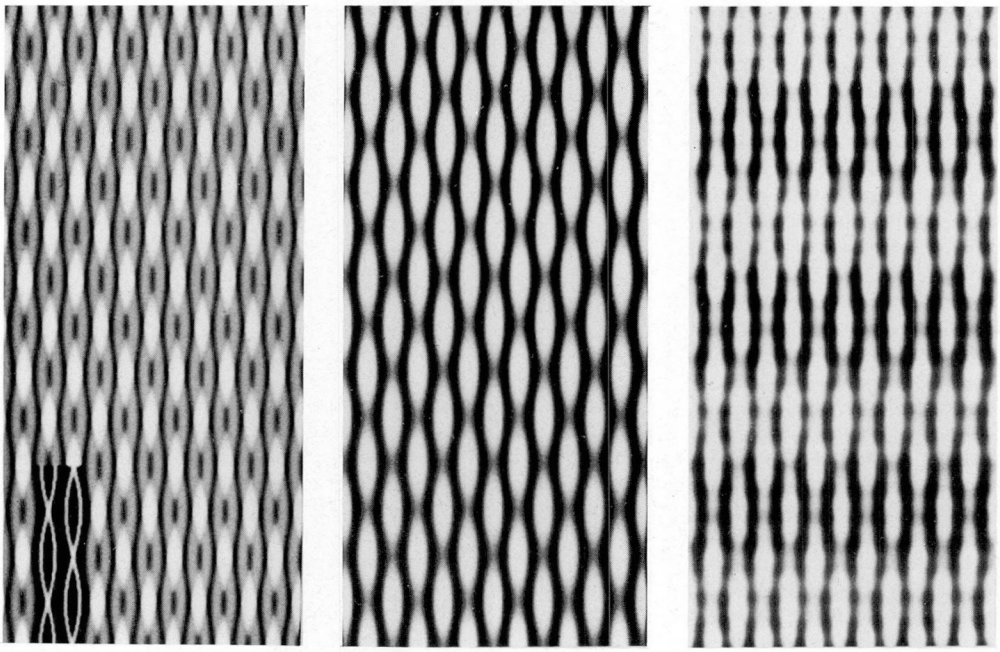

Fig. 1. Crystalline sheets of myosin long S-2 that show the coiled-coil. Schematic illustration (*left*) of the arrangement of coiled-coils in the projection of a negatively stained crystalline sheet of long S-2. Protein is shown in white and stain in black. The coiled-coils have a pitch of 14.1 nm and are spaced laterally at 2 nm intervals. Adjacent coils are staggered by a quarter pitch. A number of molecules are superimposed on one another perpendicular to the plane of the sheet, with their coils staggered by an even number of quarter pitches. The inset shows the path taken by the centre of each alpha-helix. The density is almost uniform over the array, but doubles where the coils cross over one another. The same image at a lower resolution of 1.9 nm (*centre*) shows only a pattern of light areas at the crossovers, and closely resembles the pattern seen in the image reconstructed from the electron micrographs (*right*). Reproduced from The Journal of Cell Biology, Volume 105, pp. 403–415, by copyright permission of the Rockefeller University Press.

(long S-2) to a resolution of 2 nm (Fig. 1). The sheets give diffraction patterns close to those seen by X-ray diffraction of whole muscle, and so probably give a reliable picture of both molecular structure and interaction geometry. In these sheets the pitch of the myosin coiled-coil was found to be close to 14 nm.

Because they interact over long distances, there are often long-range periodicities in coiled-coil protein sequences (Stewart *et al.* 1989). The 1000-residue myosin rod sequence contains a strong 28-residue repeat in the charged residues (Fig. 2) which produces a series of alternating positive and negative zones along the sequence. Complementation of these zones by an appropriate molecular stagger (an odd multiple of 28/2 residues) is thought to be a powerful determinant of the detailed geometry of their interaction (McLachlan, 1984). For example, the axial stagger that generates the 14.3 nm period seen in muscle and paracrystals of myosin rod fragments corresponds to a stagger of 7×28/2 residues.

In the long S-2 sheets, the coiled-coils interact in two different geometries: in the plane of the sheet, the coils are staggered by an odd number of quarter pitches, whereas perpendicular to the plane of the sheet, coils are staggered by an even number of quarter pitches. Only the second arrangement is consistent with the complementation of the alternating charged zones; the interaction in the plane of the sheets may involve the periodicities at 28/3 residues (see Fig. 2).

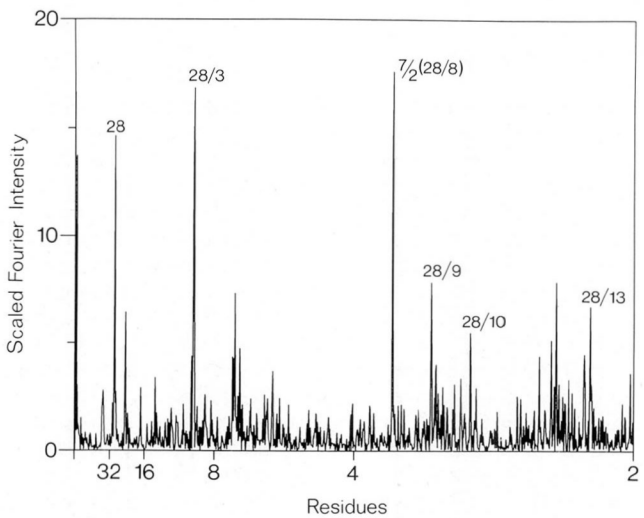

Fig. 2. Fourier transform of the distribution of the acidic residues in the nematode myosin rod sequence. The transform is calculated by first converting the sequence into mathematical form by setting acidic residues to one and all other residues to zero. The transform shows, in addition to the 7/2 and 7/3 peaks derived from the coiled-coil heptad repeat, peaks at 28 residues and overtones thereof. The peaks at 28/3 and 28/9 are particularly strong. These peaks derive from the bands of alternating charge that are thought to play an important role in the molecular interactions between myosin molecules in thick filaments.

The role of myosin rod charge periodicities

We investigated the role of the 28-residue charge periodicity in myosin assembly using a number of modified recombinant proteins produced by expressing in *E. coli* a cDNA clone corresponding broadly to the light meromyosin (LMM) region of rabbit myosin rod (Atkinson, 1990). Full-length recombinant LMM (rLMM) had solubility properties similar to proteolytic rabbit LMM and formed paracrystals with characteristic 43 nm and

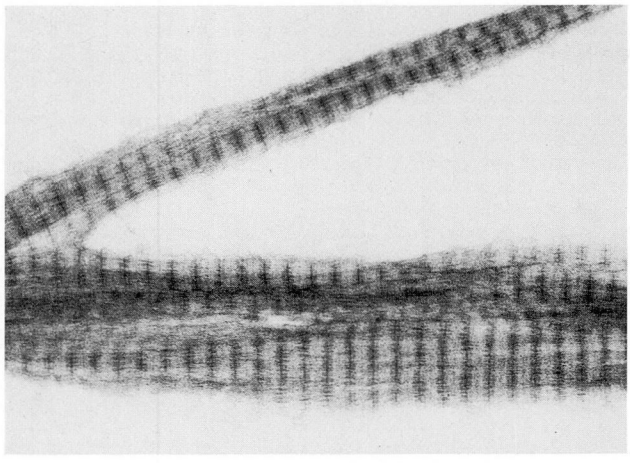

Fig. 3. Paracrystals of LMM made from recombinant material expressed in *E. coli*, formed in low ionic strength buffers and negatively stained with uranyl acetate. The paracrystals show a prominent axial banding pattern with a repeat of 43 nm. Images of metal-shadowed paracrystals confirm that the dark stripes correspond to gaps between the ends of molecules filled with stain, whereas the light bands correspond to the region in which the molecules overlap.

14.3 nm axial repeats (Fig. 3), and so clearly retained the assembly properties of native material. Several polymorphic paracrystal forms, in which the molecules were related by different staggers, were also observed to coexist in the paracrystal preparation (compare Katsura and Noda, 1973). Deletions from the N terminus of up to half the length of rLMM did not produce a marked change in its solubility properties, but deletions of as little as 90 residues from its C terminus greatly increased its solubility at low ionic strength, indicating that an important determinant of solubility is located near the rod C terminus. A similar effect has been observed with proteolytic fragments (Nyitray *et al.* 1983), but studies of deletion mutants of expressed *Dictyostelium* myosin rod (O'Halloran *et al.* 1990) indicated that in this case the solubility determining region lay about $34\times10^3\,M_r$ from the C terminus. Since the 28-residue periodicity was present throughout the rod sequence, this result indicated that complementation of the charged zones could not be the only sort of interaction important in the interaction between myosin molecules in aggregates.

The contribution of the 28-residue charged zones was evaluated more precisely using site-specific mutagenesis to construct two series of recombinant molecules containing internal deletions or insertions of 14 residues (Atkinson, 1990). These mutations change the phase of the 28-residue repeat and so prevent complementation of the charged zones over part of the region in which the molecules overlap. Remarkably, the solubility properties of these mutants were indistinguishable from those of the native material, indicating that the complementation of the zones of alternating charge made a negligible contribution to the free energy of association of the myosin coiled-coils in these aggregates. However, electron microscopy showed that the mutant proteins formed distinctly different aggregates. The wild-type protein formed paracrystals with a 43 nm axial repeat in which molecules overlapped over most of their length. The mutants formed paracrystals with a 65 nm axial repeat, in which molecules overlapped only at their ends, excluding the mutated region from the overlap, thereby avoiding unfavourable interactions involving apposition of zones of like charge. Deletions of 28 residues restored the phase of the charge repeats and yielded some paracrystals in which the 43 nm repeat and complete molecular overlap were restored.

Molecular interactions in thick filaments

The structural results obtained on vertebrate thick filaments indicate that it is unlikely that all the myosin tails in these aggregates interact in an equivalent manner. This contrasts with many other regular biological assemblies (such as virus particles, F-actin and microtubules) in which the interactions between subunits are equivalent or nearly so. However, a range of different molecular interactions is easily accommodated by the 28-residue charge repeat in the sequence, since the charged zones can be complemented by a whole range of molecular staggers. This is confirmed by the presence of polymorphic paracrystal forms. The precise molecular interaction geometries employed must await determination of the positions of the tails in the thick filament shaft. The driving force behind myosin assembly is still not completely clear, since the directed mutagenesis studies indicate that the role of the charged zones is in preventing unfavourable interactions rather than facilitating favourable ones. A number of different axial staggers appear to have very similar free energies of association. The C-terminal region of the molecule clearly has a vital role. The sequence of this part of the molecule is not remarkably different from the remainder of the rod, so it is difficult to conceive that there is a distinct myosin binding site. A concentration of positive charge in this region may be significant. Clearly further work will be required to define precisely the driving force behind myosin assembly and to identify the key interactions that determine axial and azimuthal packing of molecules. It is likely that there will be several structural levels in assembly and so different interactions may be involved in, for example, the dimerisation of molecules, the assembly of dimers into 3–4 nm protofilaments, the assembly of protofilaments into subfilaments, and the final aggregation of subfilaments into filaments. In addition, the interactions between antiparallel molecules in the bare zone will be different to those between parallel molecules in the bulk of the filaments and probably different again to the interactions at the filament tips. Mutagenised myosin molecules may be useful in disecting this hierachy of molecular interactions.

The mechanism by which *in vivo* vertebrate skeletal muscle thick filaments are precisely regulated in both length and diameter is also incompletely understood. It is difficult to account for their precise structure if these filaments arise by simple self-assembly alone, and it has not been possible to produce *in vitro* aggregates that accurately mirror the *in vivo* structure. It seems likely that some accessory proteins, perhaps analogous to those shown to be involved in virus assembly, will be required to act as a template on which the thick filament is assembled. These accessory proteins may not only regulate length and diameter, but may also specify particular interaction geometries for the incorporating monomers. Moreover, once the filaments are formed, these accessory molecules may well dissociate from the filaments and so be difficult to detect in filaments isolated from whole muscle. The

study of the molecular basis of mutant muscle phenotypes may provide the key to their identification.

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