Organization of Myosin Molecules in the Muscle Thick Filament

C. L. Davey and A. E. Graafhuis

Meat Industry Research Institute of New Zealand Inc., P.O. Box 617, Hamilton (New Zealand), 8 September 1975.

Summary. Tryptic treatment of muscle thick filaments reveals the underlying backbone of aggregated L-meromyosin as a coil of 3 secondary filaments (helical repeat ~130 nm) each in turn a coil of 3 finer ones.

The cyclical interaction of the myosin heads of thick filaments with the g-actins of the thin filaments is at the basis of muscular contraction. This interaction appears to involve subtle steric orientations of the heads and as such, the precise organization of myosin in the thick filaments is an important aspect of the contractile mechanism.

The reflections from low angle X-ray diffraction of pre-rigor vertebrate muscle are interpreted as showing that the thick filaments have their myosin-head projections arranged at successive levels of about 14.5 nm along the filament backbone. The heads are in a helical array described severally as two-stranded 6/1 of pitch 86.4 nm; four-stranded 6/1 of pitch 86.4 nm; three-stranded 9/1 of pitch 129.6 nm. The values 6 or 9 refer to the number of projections in each helical turn of each strand.

Further advance in resolving the molecular organization is likely to come from electron-microscopic identification of key details not shown by X-rays. We report pertinent new structural features revealed by controlled tryptic digestion of thick filaments to remove the H-meromyosin moiety, and to expose the underlying aggregated backbone of L-meromyosin.

Hen pectoral muscle was used to prepare natural thick filaments and the myosin for forming synthetic filaments. Digestion was carried out in two ways. Trypsin, 10 mg/ml in 0.1 M tris-HCl, pH 7.6 was added in equal volume to dilute suspensions of the filaments. After digestion, droplets of the suspensions were applied to carbon-coated grids. Alternatively the trypsin (5 mg/ml) in the same buffer was added as droplets to grids on which the filaments had already been lodged. In both cases digestion at 20°C was continued for 40 min. Head removal was incomplete at lower trypsin concentrations or shorter digestion times. Undigested and digested filaments on the grids were then fixed for 5 min in 1% glutaraldehyde, 2 mM imidazole-HCl, pH 7.0. Negative staining was carried out with 1% uranyl acetate.

Although both methods revealed the same structures, the Figure illustrates those obtained by digestion in the suspensions. The untreated natural (a) and synthetic (b) filaments, as expected, were covered in projections except for relatively smooth central regions. The roughly oblong

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a) Natural thick filaments studded with heads and showing signs of a bare central zone. × 100,000.

b) Synthetic thick filaments. Although of less than average length they are included for their clearly defined bare zones and regions of projections. In some cases the projections are cleaved, representing either the two parts of the myosin head, or head pairing. × 100,000.

c, d) The coiled backbone of fragments of natural (c) and synthetic (d) thick filaments revealed by trypsin digestion. × 200,000. The helical repeat of each strand is ~130 nm, the helix angle ~80°.

e) One of the natural backbone filaments shown in (c) further enlarged. Very clear coiling is seen by viewing it along its length at eye level. Some coiling in the secondary strands is apparent. The superimposition of the top and bottom surface patterns gives the filament a plaited appearance. × 370,000.

f, g) Portions of intertwined bundles of ~9 primary filaments produced by trypsin digestion of natural (f) and synthetic (g) filaments. These are presumed to be loosened examples of the coiled structures seen in c and d. The pieces on the right are tasselated ends of the filaments and show some fine coiling. × 100,000.

h) Proposed model of the studded regions of natural and synthetic thick filaments. The beads are the probable points of emergence of the H-meromyosin end of each molecule which are all equivalent in the structure. The beads are also a reflection of head array and take up the precise helical, three-stranded 9/1 arrangement which is one of the limited possibilities demanded by X-ray diffraction of striated muscle. In the model the helix angle is less than in the filaments. They would have been the same if cord representing the secondary strands had had the correct helix angle. Superimposing the mirror image of the model on itself would give the same sort of plaited appearance seen in (c).