MOVEMENT OF TROPOMYOSIN DURING REGULATION OF VERTEBRATE SKELETAL MUSCLE: A SIMPLE PHYSICAL MODEL

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Summary:- Using data obtained from (a) X-ray diffraction patterns of vertebrate skeletal muscle (b) three dimensional reconstructions from electron micrographs of *in vitro* aggregates of the thin filament proteins in the switched "on" and "off" positions (c) the analysis of the sequence of tropomyosin, a simple model can be proposed which may explain the geometrical arrangement of actin, tropomyosin and troponin during regulation. The "cooperative" behaviour exhibited by the thin filament can also be explained in terms of this arrangement.

X-ray diffraction patterns from contracting and relaxed vertebrate skeletal muscle show intensity differences at low angles which have been attributed to a movement of tropomyosin (TM) in the grooves of the thin filament (1-3). It was proposed by these authors that in the case of relaxed muscle ($Ca^{2+} \leqslant 10^{-6}M$), TM may sterically block the site of interaction between actin and heavy meromyosin subfragment 1 (HMM S-1). When the Ca^{2+} level increases to $\geqslant 10^{-5}M$, the calcium binds to troponin-C, one of the three chains of troponin (TN), and this affects TM in some way causing it to move away from the actin-HMM S-1 site thus allowing contraction to take place. It has not yet proved possible to explain in a simple physical manner the nature of either the forces acting on TM that cause it to move in the postulated manner or the forces which stabilise the "end" positions of TM during regulation.

Recently, three dimensional reconstructions from electron micrographs of the thin filament proteins in states corresponding to relaxed and contracting muscle (4) have independently

confirmed the X-ray analysis. In addition, it was noted that prior to muscular contraction, TM not only moves towards the centre of the groove of the thin filament but also that it appears to be more loosely bound to actin i.e. its centre of mass appears to move outwards from the surface of actin. Nonetheless, there can be little doubt that actin and TM make physical contact during the entire regulatory process.

Furthermore, it has been shown (5-7) that the sequence of α-TM (8) has a pseudo sequence repeat corresponding to the half separation of actin molecules along a long period strand of the thin filament. As the two chains of TM are in axial register (9,10) and the coiled-coil like structure of TM has a supercoil pitch length of 137Å (7,11) (measured in its own frame of reference) each actin will be regulated by TM in a pseudo equivalent manner. It also follows that actin and TM have an equivalent azimuthal relationship at their points of contact. Each actin separation corresponds precisely to $P'/_2$ (7,11,12) where P' ($\backsim$116Å) is the supercoil length of TM measured in the helical frame of reference defined by the long period grooves of the thin filament.

All this data may now be incorporated into a simple model which may describe the geometrical relationship between actin, TM and TN during regulation. Suppose that in relaxed muscle, the orientation of the TM double helix is close to the "flat on" position on the surface of each actin at their mutual points of contact (Fig. 1a). The surface area of actin thus covered is near maximal in the region of the actin-HMM S-1 binding site. In the presence of sufficiently high levels of Ca^{2+} ($\geqslant 10^{-5}$M), a strong interaction can be made between actin and the TM-TN complex. Due to the inherent asymmetry of the TM-TN complex

with respect to actin, it may be necessary for TM to rotate an angle θ about some arbitrary axis (say) P (Fig. 1a and 1b) in order to make and maximise this interaction. This rolling movement would have the effect of moving TM in towards the centre of the groove of the thin filament and also moving the centre of mass of TM out from the actin surface as observed. The angle θ cannot be estimated with any great precision from the three dimensional reconstructions but may be about 90°. The pseudo equivalent nature of the actin-TM interactions is retained axially but altered significantly azimuthally. Stewart

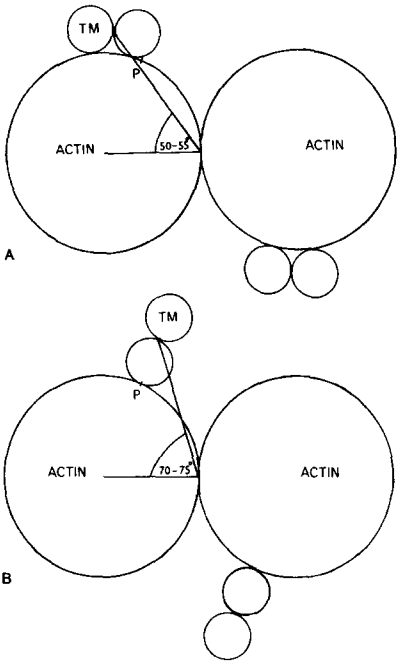

Fig. 1 A section through the thin filament corresponding approximately to the actin-TM points of contact.
(a) The postulated position of TM in relaxed muscle.
(b) The postulated position of TM in contracting muscle.
The figure is schematic and the actual angle of rotation of TM between (a) and (b) is not necessarily as indicated. The axis about which TM may rotate is indicated by P though the actual position of P is not known.

and McLachlan (7,13) have proposed that the 14 pseudo equivalent repeats noted in the linear distribution of certain groups of amino acids in the TM sequence may correspond to two sets of seven actin-TM interactions, one for the switched "off" situation and the other (or both) for the switched "on" case. The proposed rolling of TM over the actin surface could strain azimuthally one set of actin-TM contacts and optimise a second set. It should be noted that these two sets of possible actin-TM sites of interactions differ azimuthally by 90°, a value which is close to the most likely value of θ and one which may minimise the surface area of actin obscured by TM in the region of the actin-HMM S-1 binding site. A torque acting on TM could cause it to move away from the site of interaction between actin and HMM S-1 and allow contraction to take place. At a calcium concentration of less than 10^{-6}M, the strong interaction may not be maintained and TM may return to its inherently most stable "flat on" position.

The cooperative effects exhibited by the thin filament (14,15) may be explained in terms of this model. A torque about an axis approximately parallel to that of the TM molecules, whether it arises from a calcium sensitive mechanism or not, is more easily transferred axially down the head to tail assembly of TM molecules than would be a local lateral translation i.e. a sliding of a portion of TM over an actin surface. A twisting of a short length of TM from its position in the relaxed state would tend to change locally the supercoil pitch length of TM. As a consequence, actin-TM interactions adjacent to regions of local distortion could be strained both axially and azimuthally as TM attempts to return to its minimum energy state. The possibility of alternative sets of actin-TM interactions

depending on the state of the muscle is an attractive hypothesis (13) capable of explaining the stability of the "end" positions of TM in the relaxed and contracting states. The Ca^{2+} sensitised motion of TM in vertebrate skeletal muscle is, however, most likely to be the result of an interaction between TM-TN and actin.

In the case of molluscan muscle where TN is not found in the thin filaments, the movement of TM must be caused directly by the pushing effect of HMM S-1 on TM. The X-ray data from contracting muscle, interpreted by Parry and Squire (3), clearly showed that TM remains in contact with HMM S-1 and is not well displaced from the actin-HMM S-1 binding site as found in skeletal muscle. This means that the rolling movement of TM must be insufficient to optimise the alternative set of actin-TM interactions indicated by the sequence studies. As soon as the actin-HMM S-1 site is free of HMM S-1, TM may return to its energetically favoured "flat on" state.

The proposed spatial arrangement of actin, TM and TN during regulation takes into account all the known structural data. The finer details of the rolling movement cannot be indicated with any certainty and further biochemical and crystallographic studies on the constituents of the thin filament must be undertaken before a clearer understanding of the regulatory mechanism will be obtained.

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