Complexities in solution

Payne and Rudnick[1] describe evidence that the control of binding of myosin to actin occurs by mechanisms more complex than steric blockade. While this seems a real possibility, I thought it worthwhile to point out a problem with many experiments in this area, indeed in the general area of muscle contraction.

Binding of myosin to actin *in vivo* occurs after the S1 moiety of myosin collides with the appropriate available binding site on the actin filament. In the natural state the myosin is tethered at one end to the thick filament and the actin is part of the thin filament. The freedom of motion of each molecule is greatly restricted by the (nearly) macroscopic geometry. The entropy of binding of S1 to actin must be expected to be very different in the *in vivo* situation and in a free solution of soluble proteins.

The entropy of binding is an important component of the free energy in most enzymatic reactions. Thus, the rate and equilibrium constants of binding may be quite different *in vivo* from *in vitro*. Complexity in pathways would then occur in solution, if more reactions with nearly equal free energies changes occurred.

I wonder how many of the complexities observed by Payne and Rudnick, and perhaps by other workers using soluble preparations of muscle proteins, occur *in vivo*. Could one reaction be favored *in vivo* because of its reduced entropy of binding? In this way, could the geometry of the filaments, sarcomere and myofibril contribute to the rate constants of the chemical reactions of muscle?

Reference

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Further considerations of the steric blocking model

We read with interest Payne and Rudnick's recent article in *TIBS*[1] on the regulation of contraction in vertebrate striated muscle. They, quite correctly in our view, have drawn attention to the fact that the currently accepted model for the calcium regulation of muscle contraction (the steric blocking hypothesis) suffers from considerable inadequacies and is unable to offer any explanation of a large number of observations which have been made over the past two decades.

Payne and Rudnick[1] have, however, failed to consider some of the most telling data which cast doubt on the validity of the steric blocking hypothesis. These published results, based mainly on a consideration of the properties of the inhibitory protein, troponin-I (TN-I), also suggest a scheme which offers an alternative explanation of how muscle fibres respond to an increase in the concentration of free Ca^{2+} ions (from 10^{-7} M to 10^{-5} M). If actomyosin fibres (prepared from mammalian striated muscle) are washed extensively at low ionic strength the regulatory components [troponin-I, troponin-C (TN-C), troponin-T (TN-T) and tropomyosin] are removed. The ATPase activity of this preparation is appreciably reduced by the addition of the inhibitory component, TN-I[2], suggesting that troponin exerts a direct effect upon the actomyosin ATPase. The sites of interactions between TN-I and actin have been mapped with some precision – the actin binding site on the inhibitory component being located between residues 100 and 115[2,3], whilst troponin-I binds at two sites located close to the N-terminus of actin[4].

Not only does troponin-I inhibit the ATPase activity of desensitized actomyosin, but the peptide spanning the actin binding site (amino acids 96–117) is also capable of causing an appreciable reduction in enzymic activity[2]. These observations strongly support the contention that troponin-I interacts specifically with actin, and that this interaction can result in inhibition of actomyosin ATPase. The reduction in enzymic activity caused by both intact TN-I and its constituent peptides is potentiated in the presence of tropomyosin[2]. It is therefore apparent that troponin I can act as a direct inhibitor of actomyosin and that contractile activity may be regulated by a mechanism other than that propounded by Huxley and Haselgrove, or indeed by Payne and Rudnick.

On the basis of these observations, we would like to propose an alternative model to account for the regulation of contraction in vertebrate striated muscle. In the absence of calcium ions ($<10^{-7}$ M) conformational constraints on the whole thin filament (caused by tight binding of TN-I to actin monomers) may prevent strong actin–myosin interactions and ATP hydrolysis by actomyosin (due to the very slow release of P_i). The binding of Ca^{2+} to TNC at the low-affinity, calcium-specific sites within TN-C causes a conformational change such that a second interaction with TN-I can take place. The binding of TN-C to TN-I at a site very close to the actin binding site probably causes the dissociation, or at least modification, of the TN-I–actin complex. The change in actin conformation may then be transmitted along the I filament, allowing the ATP hydrolysis reaction to go to completion[5].

In this model tropomyosin could play one of two roles: its function could be (1) merely to hold the actin molecules in the correct orientation in the I filament, in which case the signal caused by Ca^{2+} influx would be transmitted directly from actin to actin, or (2) as Payne and Rudnick suggest, the tropomyosin molecule could exert a direct conformational influence on each actin in the I filament. Obviously a difficulty with all of the views of regulation is that not all actin monomers are equivalent in so far as only one in seven binds TN-I. An explanation of how the periodic location of the TN–TM complex is achieved may go some way to resolving remaining difficulties with the control of contraction.

References

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