DO TROPOMYOSIN AND MYOSIN COMPETE FOR ACTIN SITES IN THE PRESENCE OF CALCIUM?

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1. Introduction

The steric model of relaxation for actin-regulated muscles has been widely accepted because of its elegant simplicity. As proposed [1–3], the model suggests that during relaxation tropomyosin occupies the myosin binding sites on the actin filament and sterically blocks myosin attachment to actin and that when calcium binds to troponin, tropomyosin moves away from the myosin binding sites near the periphery towards the groove of the actin filament.

X-ray diffraction studies [2,4] and optical diffraction studies [5] provide evidence that tropomyosin shifts its position on the actin filament during the transition from relaxation to contraction. Also there is good evidence that, in the absence of calcium, troponin (through its subunit troponin I which binds to actin) holds tropomyosin at the periphery. Calcium saturation of troponin C initiates a series of conformational changes [6] that result in the dissociation of troponin I from actin [7] and allow tropomyosin to move to its natural binding sites at or near the groove. Although movement of tropomyosin is well established it is not clear that tropomyosin and myosin actually compete for the same binding sites on the actin filament. This point has only recently come under investigation [8,9].

While the steric model explains well the basic phenomenon of relaxation it does not provide an obvious explanation for some biochemical and physiological observations pertaining to regulation:

(i) Relaxation diminishes or disappears at low ATP concentrations [10,11].

(ii) Pure (troponin-free) tropomyosin and also calcium-saturated troponin–tropomyosin, in spite of their supposed position in the groove, are nevertheless capable of inhibiting actin activation of ATPase activity [12–14].

In this paper we: (1) show that relaxation is completely independent of ATP concentration when actin is present in large excess over S-1 (single myosin heads); (2) present data which suggest that calcium-saturated troponin–tropomyosin may still be capable of competing with myosin for actin sites although much more weakly than during relaxation (when troponin binds to actin). Some of these data have been summarized in two symposium volumes [15,16].

2. Materials and methods

All proteins and tropomyosin–actin filaments with and without troponin were prepared as in [10,12]. ATPase activity was measured in the presence of 5 mM creatine phosphate and 1–2 mg/ml creatine kinase, 1 mM Mg excess over MgATP, 10 mM imidazole (pH 7.0) and ionic strength adjusted with KCl to 35 mM; 25°C.

3. Results and discussion

3.1. *Relaxation: dependence on ATP concentration*

When regulated actin was in 100-fold excess over S-1 (fig.1) the removal of calcium caused inhibition of ATPase activity at all ATP concentrations, even at 0.5 μM ATP (fig.1, curve 3). This demonstration that very low ATP was sufficient for relaxation when S-1 was held at <2% of the actin concentration supports our view [10,11] that ATP affects relaxation

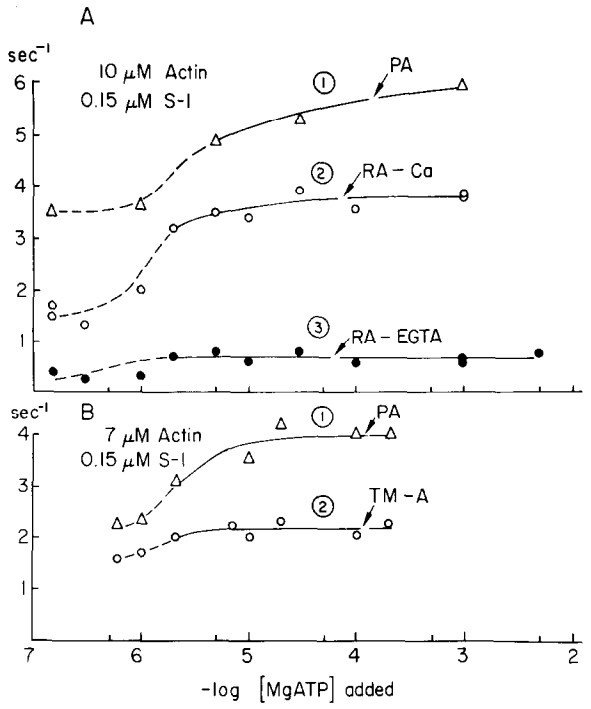

Fig.1. Acto–S-1 ATPase activity as a function of ATP when actin was in 100-fold excess over S-1. The rate of ATP hydrolysis by S-1 alone (maximal rate 0.05 s^{-1}) was subtracted. The broken lines indicate the range of ATP concentrations that are only approximate because the ATP contamination introduced with the actin, 0.5–1 μM, became significant. PA, pure actin; RA, (regulated) troponin–tropomyosin actin; TM-A, tropomyosin actin. When present: Ca was 0.1 mM $CaCl_2$ + 1 mM CaEGTA; EGTA was 1.0 mM.

only in so far as it prevents the accumulation of rigor complexes on the actin filament. (Rigor complexes are nucleotide-free actin–S-1 complexes which are normal intermediates during ATP hydrolysis by acto–S1 [19a]. They dissociate upon ATP binding to S-1.)

Rigor complexes accumulate when S-1 is in excess over actin and the ATP concentration is low; under these conditions there is no inhibition of ATPase activity and contraction occurs even in the absence of calcium (fig.3, curve 2; see also [11]). Rigor complexes may be expected to successfully compete with tropomyosin for the actin binding sites because of their relatively long lifetime at low ATP concentrations (rigor complex affinity at this ionic strength is described by K_b 1–5 × 10^7 M^{-1} [17–19].

However, the formation of rigor complexes on actin sites otherwise occupied by tropomyosin is not enough to explain shortening or activation of ATPase activity. It is necessary to have completely unoccupied actin sites capable of speeding up the release of ADP and phosphate from S-1 · ADP · P [19a,20] or in physiological terms, to which myosin bridges can attach and generate tension. The creation of such free actin sites by rigor complexes has been described as the cooperative effect of rigor complexes [10–12]. The properties of tropomyosin may provide the structural basis of this cooperativity. All tropomyosin molecules are connected by overlap sites into a long strand with a repeat unit of 41 nm for every 7 actins [21]. Assuming that tropomyosin occupies equivalent sites on every actin monomer, a tropomyosin strand of fixed length can bind closely to actin only in one position and its symmetrical counterpart [3]. The steric model of relaxation [3] requires that tropomyosin occupy that unique position during relaxation. When rigor complexes are bound to some but not all of the actin molecules and have displaced the corresponding tropomyosin segments from this unique position towards the groove the intervening tropomyosin segments can stay bound to the peripheral actin sites only if they protrude from the groove in sharp bends (fig.2A). Although a tropomyosin strand of unchanged length is too long to fit into the groove ([3] plate I) it may not be long enough to follow the path described by such bends (fig.2A) and, as we would like to suggest, is too rigid to bend sharply. As a result, the whole strand is moved towards the groove, thereby vacating the actin sites not occupied by rigor complexes. In other words we propose that the structural basis for the cooperativity of the ATPase behaviour is the fixed length and the relative rigidity of the tropomyosin strand.

3.2. *Tropomyosin inhibition in the presence of calcium*

When actin is in large excess over S-1, troponin–tropomyosin inhibit ATPase activity even in the presence of calcium [12–14] (fig.1(A) curves 1,2; (B) curves 1,2). Fig.1 shows that the inhibition by pure tropomyosin is very similar to that caused by calcium-saturated troponin–tropomyosin suggesting that after calcium saturation troponin no longer exerts as significant effect on the actin filament. This is not surprising since calcium-saturated troponin no longer binds to actin [7].

Can this tropomyosin inhibition be attributed to a

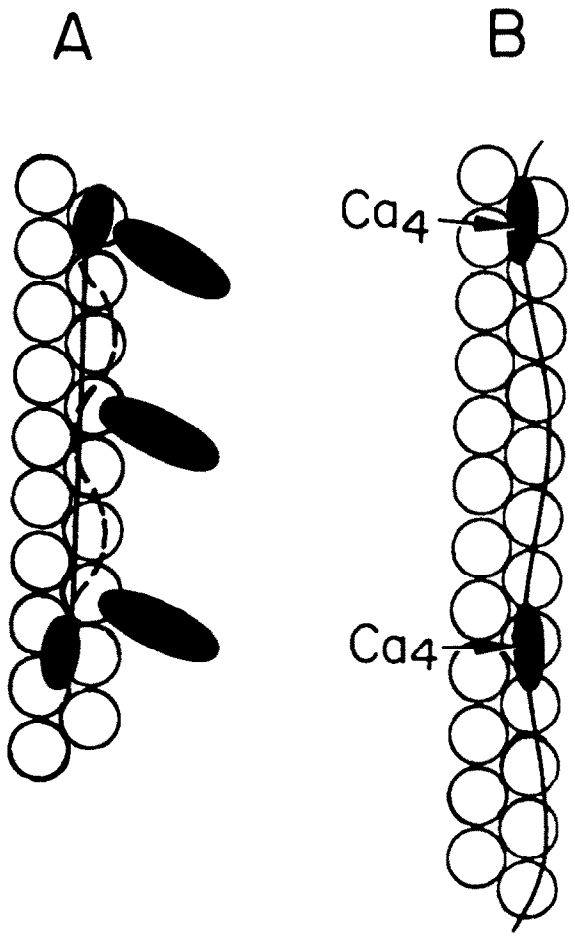

Fig.2. (A) shows a tropomyosin strand completely displaced into the groove. The broken line indicates the sharp bends that are not permitted by the relative rigidity of the molecule. (B) suggests the disposition of the tropomyosin strand in the absence of attached myosin when troponin is either calcium saturated or absent (not shown).

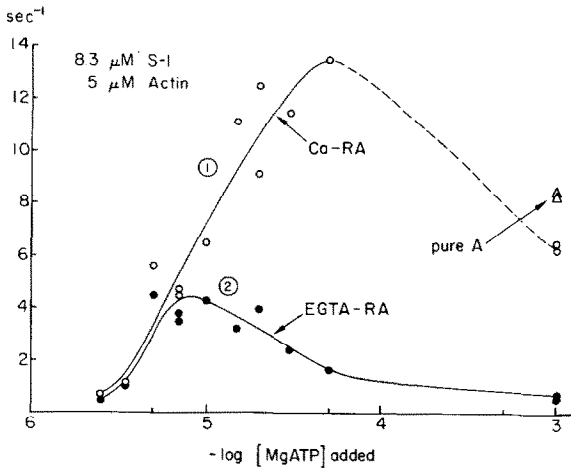

Fig.3. Activation of S-1 ATPase by regulated actin in the presence and absence of calcium (concentrations as in fig.1A) when S-1 was in excess over actin. ATPase activity in the absence of calcium increased at low ATP. In the presence of calcium, ATPase activity was biphasic and fell below that of pure actin (triangles) at 1.0 mM MgATP.

competition between tropomyosin and myosin for the same actin sites? Such a mechanism would be ruled out if kinetic analysis should reveal that tropomyosin lowers the V_{max}, i.e., inhibits the reaction step:

acto–S-1 · ADP · P → acto–S-1 + ADP + P_i

Fig.3 shows that tropomyosin did not affect V_{max} but instead increased the K_m for actin. This observation is compatible with binding site competition although it does not prove it. Competition would mean that tropomyosin occupies at least a fraction of the myosin binding sites, thereby reducing the number of actin molecules readily accessible to myosin, so that the rate of S-1 · ADP · P binding to actin is decreased.

How does one visualize the positioning of the tropomyosin strand under these conditions? The helix described by the actin sites near the groove is shorter than the tropomyosin strand so that tropomyosin does not fit into the groove. Tropomyosin may form long shallow bends between the groove and periphery, or may shift to a position intermediate between the groove and periphery, the choice between alternatives depending on which parts of the actin surface bind most strongly to tropomyosin. All that is necessary to interpret the data in terms of competition is that 1/2–2/3rds of the actin sites are not readily accessible to myosin, so that the 2–3-fold increase in the K_m for actin is accounted for. Since the affinity of tropomyosin for actin molecules is rather low [15] the position of bends would be expected to shift rapidly along the length of the filament.

3.3. *Tropomyosin activation*

If pure tropomyosin or calcium-saturated troponin–tropomyosin competes with S-1 one would expect increasing concentrations of S-1 to displace tropomyosin from the periphery, thereby raising ATPase to the same rates as in the absence of tropo-

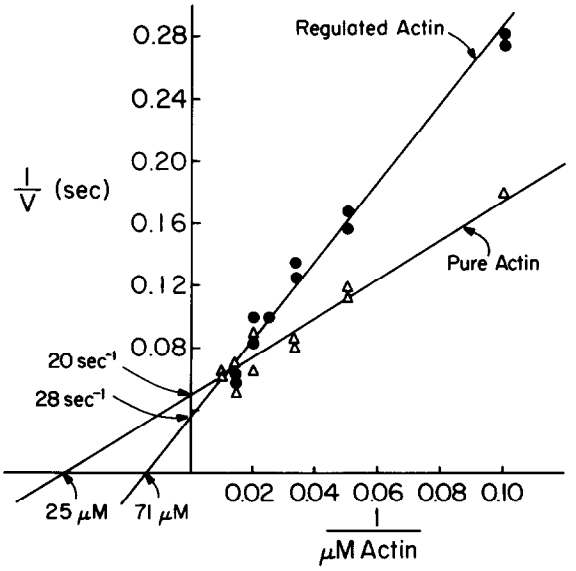

Fig.4. Double reciprocal plot of acto–S-1 ATPase activity with pure and regulated actin when actin was present in 10–100-fold excess over S-1; 0.13 μM S-1, actin 10–100 μM. Conditions as in section 2 except for 0.1 mM $CaCl_2$, 1 mM Ca-EGTA, and 1 mM MgATP.

myosin. Increasing S-1 does overcome the inhibition. However, rather than restoring activity to the rates characteristic of tropomyosin-free actin the ATPase rate is raised to a much higher value [12,13]. This is especially evident when the life time of the acto–S-1 complex is increased at low ATP concentrations. For example in fig.3, curve 1, it can be seen that the rate at 0.2 mM ATP is much higher than with pure actin at 1.0 mM ATP. We have called this phenomenon potentiation [12]. Although we suggest that potentiation is associated with complete displacement of tropomyosin into the groove the phenomenon cannot be accounted for by that alone. The fact that ATPase activity rises above that found with pure actin requires an additional cooperative structural event that depends on the interaction between groove-bound tropomyosin and myosin and actin.

In conclusion, the biochemical data are compatible with a competition between myosin and tropomyosin for the same actin sites that continues after calcium activation. However, this continued competition does not agree with the interpretation of the structural data [2,5] that even when there is no myosin attachment tropomyosin is shifted completely into the groove supercoiling to the appropriate length [3]. Since the interpretations of intensity changes of the X-ray layer lines were somewhat semiquantitative, and in view of the recent reinterpretations of the optical diffraction data of decorated actin [9], it seems possible that the structural assignments of tropomyosin position may not be final.

Nevertheless, one must keep in mind that conformational changes may contribute to the inhibition by pure tropomyosin and possibly even troponin–tropomyosin during relaxation, even though the concept of steric hindrance of myosin access under all conditions of inhibition seems more elegant.

Acknowledgements

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