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The inhibition of the adenosine triphosphatase activity of the subfragment 1–actin complex by troponin plus tropomyosin, troponin B plus tropomyosin and troponin B

In the sliding filament model of skeletal muscle[1,2] it has been proposed (for review see ref. 3) that during contraction, when the rate of ATP hydrolysis is high, the myosin cross bridges interact with the actin of the thin filaments. In the relaxed state, however, the rate of ATP hydrolysis is low and the muscle can be easily stretched and thus interaction between the thick and thin filaments is not expected. The contraction relaxation cycle is regulated by calcium, and the proteins, troponin and tropomyosin[4]. To examine the regulatory mechanism we studied the kinetics of the Mg^{2+}-activated ATPase activity of a model system in which myosin was substituted by heavy meromyosin subfragment 1 (S-1). From the kinetic data it should be possible to determine whether troponin and tropomyosin, in the absence of calcium, affect the maximum rate of ATP hydrolysis or the apparent dissociation constant of the actin–S-1 complex, or both. A kinetic analysis of the activation of the Mg^{2+}-ATPase activity of heavy meromyosin by actin has been previously reported by EISENBERG AND MOOS[5] and by SZENTKIRALYI AND OPLATKA[6], and that of the activation of S-1 by actin by EISENBERG *et al.*[7], and LOWEY *et al.*[8].

Troponin has recently been separated into two functional components, troponin A and troponin B (refs. 9, 10). The latter inhibits the Mg^{2+}-activated ATPase activity of desensitized actomyosin in both the absence and presence of calcium. The inhibition is stronger in the presence of tropomyosin. We examined the kinetics of the inhibition by troponin B and by troponin B plus tropomyosin to determine whether the inhibition by the calcium insensitive troponin B was similar to that shown by troponin and tropomyosin.

S-1 was prepared by the method of LOWEY *et al.*[8]. Troponin, troponin A, troponin B and tropomyosin were prepared as described previously[9,11]. F-actin was prepared by a method similar to the one described by TSUBOI[12]. ATPase activity was assayed in both calcium containing and calcium free systems as described previously[13]. The amount of S-1 used (about 0.1 mg/ml) was constant in each experiment and the actin concentration was varied from approx. 0.10 to 1.5 mg/ml. The molecular weight of G-actin was taken as 46000 (ref. 14).

Because actin acts as an activator, the rate of ATP hydrolysis should be related to the concentration of the actin by a Michaelis–Menten type of expression. This has been shown to be true in the cases of the activation of heavy meromyosin and S-1 by actin[5–8]. Since it has been demonstrated that the simple Lineweaver–Burk plot can result in very inaccurate values of the kinetic parameters[15], our data were analyzed by the statistical method of WILKINSON[16]. The calculations were performed on a digital computer using a modification of the program by CLELAND[17]. Any results subject to a standard error of greater than 15 % were discarded. The data in the figures are shown on Eadie plots. The intercept on the v-axis gives the value of the maximum velocity, V, expressed as μmoles P_i liberated/min/mg of S-1. From the

Abbreviations: S-1, heavy meromyosin subfragment 1; EGTA, ethylene glycol bis-(β-aminoethyl ether)-N,N'-tetraacetic acid.

slope of the graph a value of the Michaelis–Menten constant, K_{app}, is obtained. This constant is closely related to the dissociation constant of the actin–S-1 complex.

To confirm the supposition that troponin and tropomyosin are bound to actin (see ref. 4), and to show that troponin B is also bound to actin, a constant amount of troponin B and S-1, together with varying amounts of actin, were added to the assay system. If troponin B were bound to S-1, the extent of inhibition should not depend on the concentration of actin. It can be seen from Fig. 1 that this was not

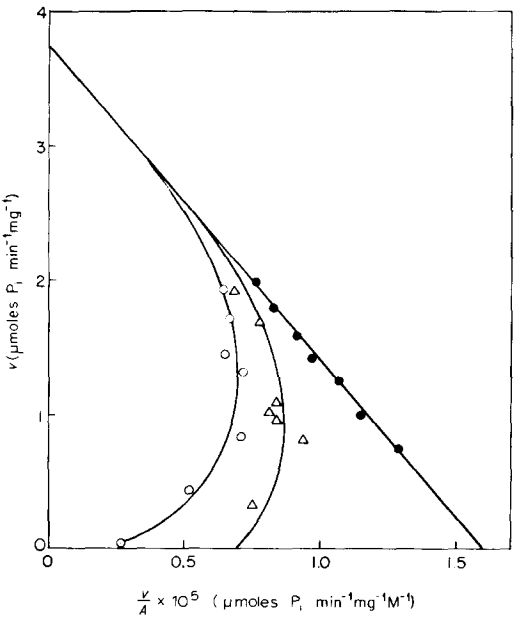

Fig. 1. Effect of a constant amount of troponin B and troponin plus tropomyosin. Assay conditions: 2.5 mM $MgCl_2$, 2.5 mM ATP, 1 mM EGTA, 25 mM Tris–HCl (pH 7.6), S-1 constant at 0.075 mg/ml. The actin concentration (A) was varied and added to 200 μg troponin B (△) and to 200 μg troponin plus tropomyosin (○) in a 1.3:1 weight ratio respectively. Control actin (●).

the case. The deviation from Michaelis–Menten kinetics at low actin concentrations is consistent with the fact that troponin B is bound to actin. Similarly the results obtained with troponin plus tropomyosin in the absence of calcium (Fig. 1) indicate binding to actin rather than S-1. Thus a constant ratio of actin to the protein to be tested was used in the following experiments.

Troponin plus tropomyosin: A mixture of troponin to tropomyosin of approx. 1.3:1 (weight ratio) was used. The Mg^{2+}-activated ATPase activity was measured in the absence of calcium (*i.e.* in the presence of EGTA) for ratios of actin:troponin plus tropomyosin of 1:0.075 and 1:0.1. It can be seen from Fig. 2 that the values of V (5.1 and 5.3) are similar to the value of V of 5.2 obtained in the control experiment in which troponin and tropomyosin were absent. There is, however, a considerable difference between the K_{app} value of $2.8 \cdot 10^{-5}$ M obtained for the control experiment and the values of $4.5 \cdot 10^{-5}$ M and $4.8 \cdot 10^{-5}$ M obtained for the actin:troponin plus tropomyosin ratios of 1:0.075 and 1:0.1, respectively. It may be proposed therefore that in the absence of calcium, the inhibition of the Mg^{2+}-activated ATPase activity by troponin plus tropomyosin is due to an increase in the dissociation of the actin–

S-1 complex. Similar results were obtained when actin containing troponin and tropomyosin (*i.e.* impure actin) was used. Recently, EISENBERG AND KIELLEY[18] have also found that troponin and tropomyosin effect a shift in K_{app} rather than in V.

Troponin B plus tropomyosin: Since in this system the inhibition of the Mg^{2+}-activated ATPase activity is essentially calcium-insensitive, the assays were performed in the presence of calcium. The results, shown in part in Fig. 3, again indicate similar values of V for the control and test systems with a marked difference in the K_{app}

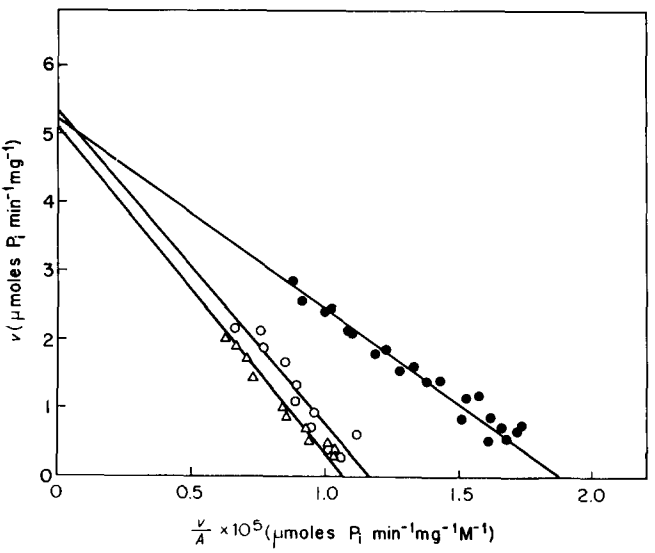

Fig. 2. Effect of constant ratios of actin:troponin plus tropomyosin. Assay conditions as in Fig. 1. S-1 constant at 0.1 mg/ml. The actin concentration (A) was varied. The following actin:troponin plus tropomyosin ratios were used: 1:0.075 (○), 1:0.1 (△). The troponin and tropomyosin were present in a 1.3:1 weight ratio respectively. Control actin (●).

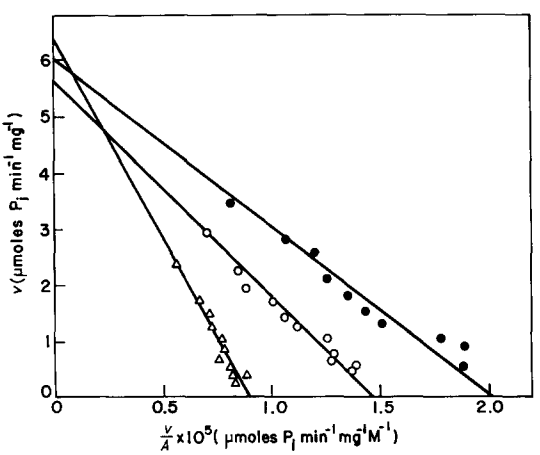

Fig. 3. Effect of constant ratios of actin:troponin B plus tropomyosin and constant ratios of actin:troponin B. Assay conditions: 2.5 mM $MgCl_2$, 2.5 mM ATP, approx. 10^{-5} M $CaCl_2$, 25 mM Tris–HCl (pH 7.6), S-1 constant at 0.1 mg/ml. The actin concentration (A) was varied. Actin: troponin B plus tropomyosin, 1:0.075 (○); actin:troponin B, 1:0.2 (△); control actin (●).

values. Values of 6.0 for V and $3.0 \cdot 10^{-5}$ M for K_{app} were obtained for the control. V values of 5.6 and 6.0, and K_{app} values of $3.8 \cdot 10^{-5}$ M and $5.0 \cdot 10^{-5}$ M were obtained for the actin: troponin B plus tropomyosin ratios of 1:0.075 and 1:0.15, respectively. The troponin B and tropomyosin were present in equal proportions.

Troponin B: These assays were once again conducted in the presence of calcium. As indicated in Fig. 3 the inhibition of ATPase activity by troponin B resulted in little change in V (6.0 for the control and 6.4 in the presence of troponin B) and an appreciable change in K_{app}. The values of K_{app} are $3.0 \cdot 10^{-5}$ M for the control and $5.6 \cdot 10^{-5}$ M and $8.0 \cdot 10^{-5}$ M for the actin:troponin B ratios of 1:1.15 and 1:0.2, respectively.

The above data suggest that troponin and tropomyosin, in the absence of calcium, reduces the extent of interaction between myosin and actin. It is also apparent that troponin and tropomyosin do not alter the activating ability of actin, since at infinite actin concentrations V was constant. It has been suggested that the effect of calcium is to induce a conformational change in troponin which can be transmitted through tropomyosin to actin, with the result that the actin–myosin interaction is modified[19,20]. If this is the case, then it appears likely that these changes allow a reversible blocking of the site on actin for myosin interaction without significantly modifying the properties of actin.

The results obtained with troponin B plus tropomyosin and troponin B alone indicate that the nature of the inhibition is similar to that obtained with troponin and tropomyosin in the absence of calcium.

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