Troponin I and troponin T interact with troponin C to produce different Ca^{2+}-dependent effects on actin–tropomyosin filament motility

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We have developed an *in vitro* motility assay to make a detailed quantitative analysis of Ca^{2+} control of skeletal-muscle troponin–tropomyosin control of actin-filament movement over immobilized myosin. Ca^{2+} regulates both filament velocity and the fraction of filaments that are motile. We have demonstrated that the two effects are due to separate interactions of troponin C with troponin I and troponin T. When 64 nM of the complex actin–tropomyosin–troponin I–troponin C was added at pCa 5, more than 80 % of filaments were moving and their velocity did not change. At pCa 9, more than 20 % of the filaments were moving. When 20 nM of the complex actin–tropomyosin–troponin T + troponin I + troponin C was added at pCa 5, filament motility remained high, whereas velocity increased. The 30 % increase in velocity observed when troponin T was present was also observed when heavy meromyosin fragment 1 labelled with *N*-ethylmaleimide (NEM S-1) was added after actin–tropomyosin filaments. The NEM S-1 effect was not additive with the troponin T-dependent velocity increase. The pattern of motile behaviour is characteristic of myosin on silicone-treated glass and different from the behaviour on nitrocellulose-coated glass.

INTRODUCTION

The Ca^{2+} regulation of actomyosin by troponin and tropomyosin has been studied intensively over the last 25 years, and a generally accepted mechanism for control of contractility has emerged [1,2]. Tropomyosin plays a central role in regulation. The effect of troponin at low Ca^{2+} concentrations is to induce a 'switched off' state of actin–tropomyosin in which myosin-head access to the 'strong' myosin-binding sites on actin is prevented, thereby blocking the cross-bridge cycle. The actin–tropomyosin state is controlled by the inhibitory component of troponin, troponin I, which acts as an allosteric inhibitor, and this effect is regulated in response to Ca^{2+} via the Ca^{2+}-binding component of troponin, troponin C [3,4].

In vitro, troponin regulates the V_{max} of actin–tropomyosin activated myosin Mg^{2+}-ATPase, whereas the K_m is virtually independent of the degree of activation [5]. In intact striated muscles, Ca^{2+} controls the force rather than the unloaded velocity [6], but Ca^{2+} also controls a kinetic step (K_{tr}), which is thought to be a measure of the rate of the 'weak'-to-'strong' transition [7]. Thus troponin may be capable of affecting the cross-bridge cycle at more than one point. This cannot be determined from ATPase assays, which measure a bulk property, but analysis of the performance of individual contractile filaments could provide the opportunity to elucidate the mechanism with sufficient molecular detail to account for the performance of intact muscle.

We have developed the *in vitro* motility assay devised by Kron and Spudich [8] to make a detailed quantitative analysis of skeletal-muscle troponin/tropomyosin control of actin-filament movement over immobilized myosin [9]. In the *in vitro* motility assay we can measure a number of parameters in place of the single parameter of ATPase: the fraction of actin filaments moving, the velocity of the filaments that are moving and the number of filaments attached to myosin per unit area.

In the absence of troponin, up to 90 % of filaments are moving, with an average velocity of 3.5 μm/min. The most striking effect of troponin is that it appears to regulate actin–tropomyosin filaments as a single unit [9,10]. At pCa 9.0, addition of up to 4 nM troponin causes the proportion of motile filaments to decrease from > 85 % to 20 %, with no dissociation of the filaments from the myosin surface or change in velocity. Increasing the Ca^{2+} concentration causes the filaments to be switched back on, with a half-maximal increase in the proportion of motile filaments at pCa 5.8–6.0 and a 30 % increase in filament velocity. When we added troponin I + troponin C, the filaments were switched on by Ca^{2+}, but there was no increase in velocity [11].

In ATPase assays it can be demonstrated that troponin I and troponin T have separate Ca^{2+}-dependent effects, both of which are mediated by troponin C [12]. Troponin I + C inhibits actin–tropomyosin activation of myosin Mg-ATPase at pCa 9, but has no effect on ATPase at pCa 5. However, when troponin T is present in addition, there is an increase in ATPase activity up to 2.5-fold at pCa 5.

On the basis of these results, we considered it possible that troponin I is responsible for controlling the fraction of motile filaments in the motility assay, whereas troponin T is responsible for the increase in velocity at high [Ca^{2+}]. This was tested by measuring the effect of reconstituted troponin I + C and troponin I + C + T complexes on the motility of actin–tropomyosin filaments. We found that troponin I + C switches off motility at pCa 9 but has no effect on velocity, whereas at pCa 5 it has no effect upon velocity or the fraction of motile filaments. In contrast, troponin I + C + T give results that are like whole troponin. Thus the motility assay can distinguish separate functional effects due to troponin I and T.

METHODS

Protein preparations

Rabbit skeletal-muscle actin, myosin, heavy meromyosin (HMM) and heavy meromyosin subfragment-2 (S-2) were prepared by

Abbreviations used: ϕ, rhodamine-phalloidin,; actin-ϕ, actin labelled with rhodamine-phalloidin; DTT, dithiothreitol; FITC, fluorescein isothiocyanate; HMM, heavy meromyosin; NEMS-1, heavy meromyosin subfragment-1 labelled with *N*-ethylmaleimide; S-2, heavy meromyosin subfragment-2.

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standard methods [13,14]. F-actin was labelled with rhodamine-phalloidin (actin-ϕ) and HMM was pre-spun in the presence of actin and ATP to remove rigor heads as described previously [9].

Tropomyosin and troponin were extracted from 200 g of rabbit skeletal muscle or sheep heart muscle ethanol/ether-dried powders according to the method described by Potter [15]. Tropomyosin was purified by repeated isoelectric precipitation, followed by chromatography on hydroxyapatite using a phosphate gradient (10–200 mM) [16]. Troponin was isolated from rabbit skeletal muscle and sheep cardiac muscle [15]. Troponin I, troponin C and troponin T were separated by chromatography of troponin on a DEAE-Fast-flow (Pharmacia) column (2.5 cm × 20 cm) in 6 M urea/50 mM Tris/HCl (pH 8.0)/1 mM EGTA/2 mM dithiothreitol (DTT) eluted in 1 litre of a 10–300 mM NaCl gradient. Heavy meromyosin subfragment-1 labelled with *N*-ethylmaleimide (NEMS-1) was prepared by the method of Swartz and Moss [17] and characterized as described by Marston et al. [18].

Troponin I+C and I+C+T complexes were made by dissolving the components at 25 μM in 6 M urea/1 M KCl/0.1 mM $CaCl_2$/10 mM Mes/5 mM DTT (pH 6.0). The mixtures were then dialysed against 1 M KCl/0.1 mM $CaCl_2$/10 mM Mes/5 mM DTT (pH 6.0) and finally slowly dialysed into the buffer used for the motility assay.

The optimum proportion of troponin I/C/T was determined in ATPase assays. Concentrations of troponin I+C and troponin I+C+T complexes are expressed in terms of the troponin I concentration.

Motility assay

In vitro motility assays were performed as described by Fraser and Marston [9] using 100 μg/ml skeletal-muscle HMM on cover glasses coated with silicone by soaking in 0.2 % (v/v) dichloromethylsilane in chloroform. In some experiments the cover glass was coated with nitrocellulose [19]. Skeletal-muscle F-actin was labelled with rhodamine-phalloidin (ϕ) as described by Kron et al. [19]. F-actin–ϕ–tropomyosin and actin–ϕ–tropomyosin–troponin complexes were formed at 10× assay concentration: 100 nM F-actin–ϕ, 150 nM skeletal-muscle tropomyosin and 0–600 nM troponin were mixed with 50 mM KCl/25 mM imidazole/HCl/4 mM $MgCl_2$/1 mM EDTA/5 mM DTT/BSA (0.5 mg/ml, pH 7.4) (buffer B) and incubated for 30–60 min. The complexes were diluted 10-fold immediately before infusion into the motility cell.

A flow cell was prepared from a freshly siliconized coverslip and a microscope slide, as described by Kron et al. [19]. Assay components and buffers were infused into the flow cell at 30–60 s intervals. Two 50 μl aliquots of HMM at 100–200 μg/ml were infused in buffer A (50 mM KCl/25 mM imidazole/HCl/4 mM $MgCl_2$/1 mM EDTA/5 mM DTT, pH 7.4) to provide a coating of immobilized HMM on the coverslip. This was followed by 2 × 50 μl of buffer B (A + 0.5 mg/ml BSA), then 2 × 50 μl of 10 nM actin-ϕ ± associated tropomyosin–troponin in buffer A. Buffer C [50 μl; B + 0.1 mg/ml glucose oxidase/catalase (0.02 mg/ml)/glucose (3 mg/ml)/0.5 % (w/v) methylcellulose ± troponin at assay concentration] and buffer D (50 μl; C + 1 mM ATP) were then infused. [Ca^{2+}] was varied by incorporating Ca^{2+}/EGTA buffers in the final assay buffers C and D.

The movement of actin-ϕ–tropomyosin filaments over the immobilized skeletal-muscle HMM was observed under a Zeiss Epifluorescence Microscope (63 × /1.4 objective) with a DAGE-SIT-68 camera and recorded on video tape. We only collected data from preparations in which at least 70 % of actin-ϕ or actin-ϕ–tropomyosin filaments were motile. Videos were digitized and the movement was analysed to determine 300–1000 filament vectors using the automatic tracking programme described by Marston et al. [20]. Frequency histograms of the vectors show that the filaments always consist of just two populations, one immobile and one moving. The moving-filament population distribution fitted a Gaussian curve with a standard deviation in the range 0.7–1 μm/s. The fraction of total filaments in the motile population and their mean velocity were determined.

ATPase and binding assays

Reconstituted troponin complexes and NEMS-1 preparations were tested for their ability to regulate ATP hydrolysis in 100 μl samples of 1 μM skeletal-muscle HMM/10 μM actin/3 μM tropomyosin in buffer A at 30 °C. The reaction was started by adding ATP to 5 mM and stopped after 15 min by adding 500 μl of 10 % (v/v) trichloroacetic acid. The P_i released was measured. Troponin I and troponin C were reconstituted at several different ratios and we observed that an excess of troponin C was needed in order to obtain full neutralization of troponin I inhibition of ATPase in the presence of Ca^{2+}. Accordingly we adopted a molar ratio of 1 troponin-I:3 troponin-C:1.2 troponin-T for reconstitution of troponin.

The composition of thin filaments used in the motility assay was determined by sedimenting the thin filaments and SDS/PAGE analysis of the pelleted material [9,11]. Tropomyosin and the troponin subunits were found to bind to actin in the same ratios as in filaments reconstituted with whole troponin, and this binding was independent of [Ca^{2+}].

RESULTS

In initial experiments we used whole troponin complex from rabbit skeletal and sheep cardiac muscle to assess the regulation of rabbit skeletal actin-ϕ–tropomyosin-filament movement. The motile filaments formed a single population with a Gaussian distribution of velocities under all conditions of motility assay. When troponin was added at pCa 5, there was a 20 % increase in filament velocity for skeletal-muscle troponin and 17 % for cardiac muscle troponin (mean values from three experiments each, Table 1), with no change in the fraction of motile filaments. At pCa 9, troponin decreased the fraction of motile filaments from > 80 % to < 20 %, as has been observed previously [9]. There was also a slight decrease in velocity, which was greater with cardiac- than with skeletal-muscle troponin (Table 1).

Troponin I–troponin C complex

We then studied the effect of the skeletal-muscle troponin I+C complex upon actin-ϕ–tropomyosin filament movement (Figure 1, panels B and D). At pCa 9, the addition of troponin I+C resulted in a large decrease in the fraction of filaments that were motile from an average of 81 % to 23 % with 64 nM troponin I+C (four separate experiments). The concentration of troponin I+C needed to 'switch off' filament movement was greater than for whole troponin, probably a consequence of weaker binding to actin–tropomyosin. The filament velocity at pCa 9 declined slightly (9 %, see Table 1) in the three experiments where velocities were directly compared. Concentrations of troponin I+C above 64 nM caused bundling of the actin. At pCa 5, troponin I+C had no effect upon either the fraction of motile filaments, which remained high, or their velocity. Co-sedimentation measurements showed that troponin I+C was bound to actin–tropomyosin under these conditions.

Table 1 Effects of troponin complexes on actin-ϕ–tropomyosin filament velocity

The movement of actin-ϕ–tropomyosin filaments over immobilized HMM was analysed. There were two populations of filaments, motile (velocity 3.5–4.5 μm/s) and stationary. Motile filaments were 80–90% of total at pCa 5 and $<$ 40% of total at pCa 9 in the presence of troponin. The velocity of the motile filaments in the presence of 4–32 nM troponin (8–64 nM troponin I+C) is expressed relative to velocity in the absence of troponin. Means and S.E.M. of relative velocity from n measurements with three to four separate protein preparations are presented.

		pCa5			pCa 9		
	Number of preparations	Change in velocity (%)	Standard error	n	Change in velocity (%)	Standard error	n
Skeletal troponin I+C	4	0%	±1.6%	11	−9%	±2%	10
Skeletal troponin I+C+T	4	+21%	±1%	10	−4%	±2%	11
Skeletal troponin	3	+20%	±2.3%	9	−12%	±7%	7
Cardiac troponin	3	+17%	±3%	5	−21%	±2%	7

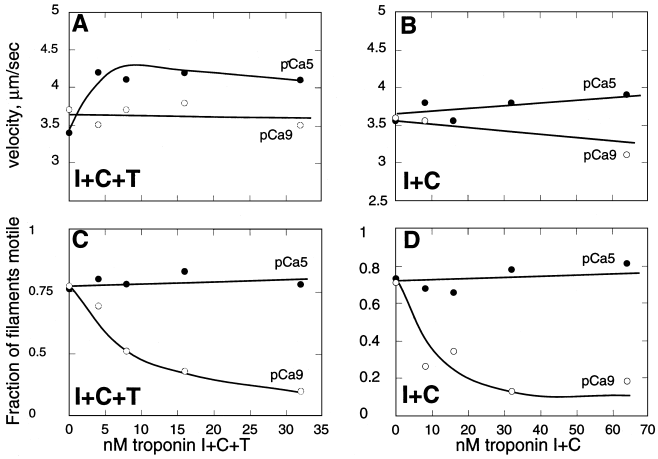

Figure 1 Effects of troponin I+C and troponin I+C+T on actin-ϕ–tropomyosin filament motility over skeletal HMM

Conditions were 10 nM actin–tropomyosin, 0–50 nM troponin and buffer A at 28 °C. Troponin complexes were reconstituted in molar ratios 1 troponin I:3 troponin C:1.2 troponin T and mixed with actin–tropomyosin before dilution to assay concentration. Troponin was also included in buffers C and D. ●, pCa 5; ○, pCa 9. (**A**) Effect of troponin I+C+T on filament velocity; (**B**) effect of troponin I+C on filament velocity; (**C**) effect of troponin I+C+T on fraction of motile filaments; (**D**) effect of troponin I+C on fraction of motile filaments.

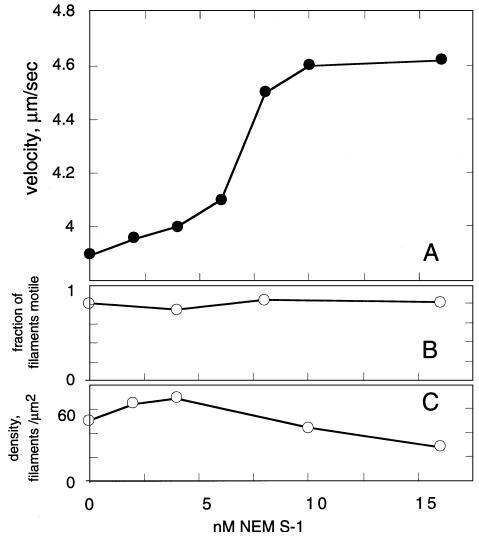

Figure 2 Effect of NEMS-1 on actin-ϕ–tropomyosin motility

Conditions were as for Figure 1. NEMS-1 was included in buffers C and D after actin-ϕ–tropomyosin had been infused into the motility cell. Filament movement was recorded and analysed for velocity of motile filaments (**A**), fraction of filaments that are motile (**B**) and density of attached filaments (**C**). NEMS-1 only enhances velocity.

Troponin I–troponin C–troponin T complex

When we reconstituted the whole troponin complex from purified subunits, we found that it regulated actin-ϕ–tropomyosin filament movement in the same way as intact troponin (Figure 1, panels A and C). At pCa 5, addition of troponin I+C+T resulted in an increase in the velocity of filaments by an average of 21 % (four separate determinations), the increase being optimal at 4–16 nM troponin I+C+T; there was no change in the fraction of motile filaments. Thus the increase in velocity due to troponin at pCa 5 must be due to the troponin T component. At pCa 9, troponin I+C+T decreased the fraction of motile filaments without significantly changing the velocity of the filaments that remained motile in the same way as troponin or troponin I+C (Table 1).

Effect of NEMS-1 on filament motility

NEMS-1 is known to have the ability to 'switch-on' actin–tropomyosin filaments by acting as a rigor cross-bridge, which

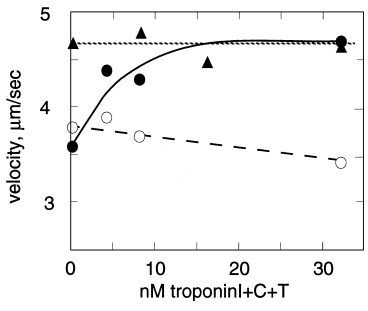

Figure 3 Effect of troponin I+C+T on filament velocity in the presence and absence of NEMS-1

Conditions are as for Figure 1, with the addition of NEMS-1 in buffer C and D as in Figure 2. ○, troponin I+C+T at pCa 9; ●, troponin I+C+T at pCa 5; ▲, troponin I+C+T at pCa 5 plus 18 nM NEMS-1. Both NEMS-1 and troponin enhance velocity, but the effects are not additive.

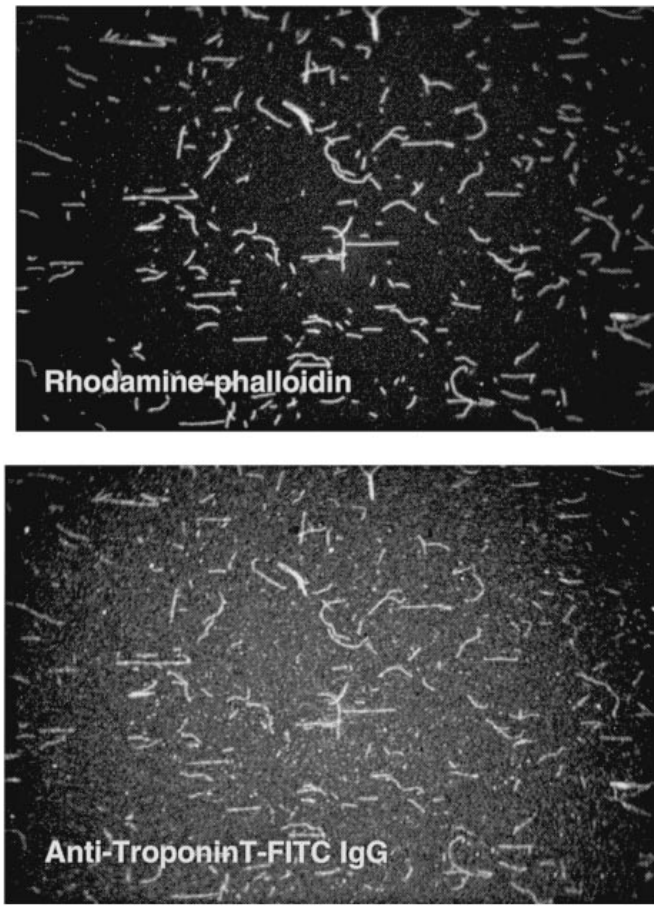

Figure 4 Double labelling of filaments for actin and troponin T

Actin-ϕ–tropomyosin (10 nM) + troponin (2 nM) at pCa 9 were infused into the motility cell and the filaments attached to HMM by rigor links. The cell was then infused with anti-troponin T monoclonal antibody diluted $\times 50$ (Sigma), followed by FITC-conjugated anti-mouse IgG. Actin-ϕ fluorescence was observed through a rhodamine filter set and troponin fluorescence was observed through a fluorescein filter set. In controls in which the anti-troponin T was omitted, there was no detectable filament fluorescence. Images were visualized with the DAGE-SIT68 camera, recorded via a Neotech Imagegrabber and printed using Neotech version 2.1 software. It was found that anti-troponin T labelled all the filaments uniformly, although at least half of them should have been switched off under these conditions (see Figure 1C).

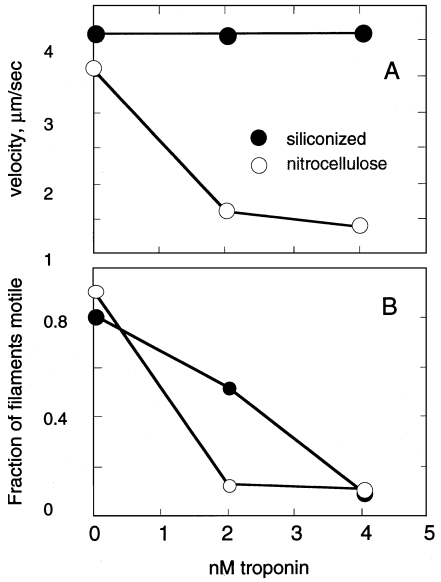

Figure 5 Comparison of the effect of troponin on actin-ϕ–tropomyosin motility using HMM coated on siliconized or nitrocellulose surfaces

Conditions as for Figure 1; whole rabbit skeletal-muscle troponin was used. Effect of troponin at pCa 9 on (**A**) velocity, (**B**) fraction, of motile filaments. At 2 and 4 nM troponin on the nitrocellulose surface, the fraction of motile filaments was too low for accurate automatic analysis [17], so motile filaments were tracked manually as described by Fraser and Marston [9].

can potentiate actomyosin ATPase activity and muscle-fibre force production via tropomyosin, even in the presence of saturating ATP. In ATPase assays, NEMS-1 enhanced actin–tropomyosin Mg-ATPase activity by up to 6-fold. Potentiation became saturated at NEMS-1/actin ratios of 0.2–0.3. When NEMS-1 was added to the motility cell after actin-ϕ–tropomyosin, it increased the filament velocity by 21, 33, 35 and 40% in four experiments using two NEMS-1 preparations. There was no significant change in the fraction of motile filaments (Figure 2). As expected, the NEMS-1 effect was wholly dependent upon tropomyosin, and NEMS-1 had no effect on velocity or the fraction of pure actin-ϕ filaments that were motile. Thus 'switching on' of actin-ϕ–tropomyosin by NEMS-1 results in an increase in filament velocity, which correlates with the increase in ATPase activity in the test tube.

The effects of troponin T and of NEMS-1 on actin-ϕ–tropomyosin filament velocity were the same (Figures 1A and 2). To determine whether they have a common mechanism, we added troponin or troponin I + C + T at pCa 5 to actin-ϕ–tropomyosin filaments that had been 'switched on' by NEMS-1. There was no further increase in filament velocity (Figure 3).

Distribution of troponin on actin-ϕ–tropomyosin filaments

One explanation for the all-or-none nature of the 'switch off' of filament movement by troponin at pCa 9 could be co-operative binding of troponin, which would result in filaments being either fully occupied or bare of troponin. The location of troponin on the actin-ϕ–tropomyosin filaments was determined by labelling troponin with anti-troponin T antibody and a fluorescein isothiocyanate (FITC)-labelled second antibody in the motility cell. With saturating concentrations of troponin (10 nM) all the actin-ϕ filaments detectable by rhodamine fluorescence were also revealed by FITC fluorescence from troponin. Moreover, at a concentration of troponin where only 30% of the filaments should be moving, the troponin fluorescence was less bright but was still uniformly present on all the filaments (Figure 4); thus there is no evidence for co-operative troponin binding.

Effect of surface coating and HMM concentration on filament motility

We have used siliconized glass surfaces to bind myosin, whereas other workers have used nitrocellulose. On nitrocellulose surfaces it is commonly reported that troponin slows down filaments at pCa 9, as well as switching them off [21]. When we compared siliconized and nitrocellulose surfaces using the same HMM, actin, tropomyosin and troponin, we did observe a decrease in velocity on nitrocellulose (Figure 5). Clearly then, the nature of the surface on which HMM is bound has a significant influence on the way troponin affects the parameters of motility.

We also examined the effect of myosin head concentration on actin-ϕ–tropomyosin filament movement. Siliconized cover

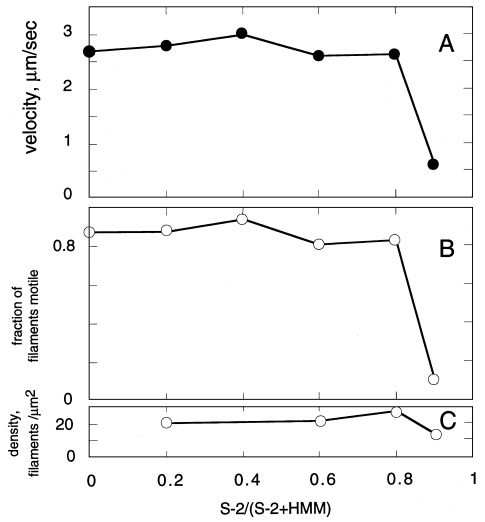

Figure 6 Effect of HMM concentration on actin-ϕ–tropomyosin filament movement

Siliconized cover glasses were coated with an HMM/S-2 mixture at a total concentration of 100 μg/ml; the fraction of HMM in the mixture was varied. The movement of 10 nM actin-ϕ–tropomyosin filaments over these surfaces was observed at 28 °C and analysed by both manual and automatic tracking techniques. The Figure shows the result of manual tracking, which is more accurate when the fraction of motile filaments becomes very small [19]. Velocity and fraction of motile filaments remained constant as HMM concentration decreased until the mixture was $>$ 80% S-2 (HMM head concentration $<$ 20 μg/ml).

glasses were coated with a mixture of skeletal-muscle HMM and S-2 (the tail part of HMM) at a total concentration of 100 μg/ml. As the proportion of HMM in the mixture decreased, the velocity and fraction of moving filaments stayed constant down to 20 μg/ml HMM. With further dilution of heads, the filaments suddenly stopped moving. At 10 μg/ml HMM, only 10% of filaments could be found to move and those that did had a very low velocity, although they remained attached to HMM and S-2 (Figure 6). A similar pattern of results was observed by simply reducing the HMM concentration, except that the density of actin-ϕ–tropomyosin filaments became very low at the lower HMM concentrations, and this prevented quantitative analysis.

DISCUSSION

The *in vitro* motility assay allows us to study the behaviour of individual thin filaments moving over immobilized myosin. Filament movement may be characterized by three parameters: the fraction of filaments that are moving, the velocity of the moving filaments and the number of filaments attached to the myosin surface per square micrometre. These variations seem to be related to important aspects of the cross-bridge cycle [9].

We have already demonstrated that skeletal-muscle troponin/tropomyosin can control all three of these parameters independently, in response to changes in [Ca^{2+}] [9], and in this paper we demonstrate that the individual subunits of troponin have distinct functional roles in controlling motility. Ca^{2+} controls the fraction of actin–tropomyosin–troponin filaments that are motile, and we can now see that this property is exclusively due to troponin I and troponin C interacting with actin–tropomyosin. Ca^{2+} also controls filament velocity, and this is shown to be due to the presence of troponin T and its interaction with troponin C, since no such effects are observed in the absence of troponin T (Figure 1).

These results are strongly in support of Potter et al.'s assignment of a regulatory role for the troponin T–troponin C interaction, in addition to the well-established troponin I–troponin C interaction [12]. Moreover, since the motility assay can detect separate effects upon the fraction of motile filaments and velocity parameters, it is possible to assess the individual regulatory properties of troponin I and troponin T in complex mixtures such as whole troponin or native thin filaments. An example of this approach to analysis of regulation has been published by Lin et al. [22], who demonstrated that a point mutation of troponin T resulted specifically in an increase in filament velocity at pCa 5.

Our observations could be accommodated by the 'three-state' model [1] formulated by McKillop and Geeves [23,24], which proposes an equilibrium between 'blocked' and 'closed' states of actin–tropomyosin controlled by troponin, and the conversion of the 'closed' state into an 'open' state by myosin heads. It is proposed that only cross-bridges attached to the 'open' state may cycle, hydrolyse ATP and produce movement. The transition from the 'closed' to the 'open' state may correspond to the increase in velocity induced by myosin heads when NEMS-1 is added, and thus suggests that troponin T may influence this step, whereas the equilibrium between 'blocked' and 'closed' states seems to equate with the 'switch off' state of motility controlled by troponin I.

Studies in skeletal- and cardiac-muscle fibres have indicated that increasing Ca^{2+} leads to recruitment of cross-bridges, but at the same time there is activation of a kinetic step, measured as K_{tr}, which is believed to represent the rate of the 'closed' to 'open' cross-bridge transition ('weak' to 'strong' transition in the formulation of Hill et al. [25]) [7,26–29]. It is an intriguing possibility that the recruitment of cross-bridges could be controlled by troponin I and the increase in K_{tr} could be controlled by troponin T. In the case of troponin T there is some positive evidence, since we observed that the increase in filament velocity due to troponin T at high [Ca^{2+}] could also be obtained by adding NEMS-1 to actin–tropomyosin filaments, indicating that the troponin T effect was likely to be due to switching tropomyosin to the fully 'open' state. The experiments of Swartz and Moss [17] show that NEMS-1 addition to muscle fibres leads to increases in K_{tr} that parallel those due to increasing Ca^{2+} and are thus compatible with a link between the kinetic step measured by K_{tr} and the troponin T–troponin C interaction. Since the control of the fraction of filaments moving by the action of tropomyosin, troponin I and troponin C seems to be an equivalent *in vitro* to the recruitment of cross-bridges when muscle is activated by Ca^{2+}, this phenomenon deserves further study.

In our system, rabbit skeletal-muscle troponin I controls motility almost entirely by controlling the fraction of filaments moving, with little effect upon the velocity of the filaments that remain motile. Other workers have observed both 'switch off' and filament slowing, and it is therefore necessary to account for the differences [21,30]. A comparison of results with the widely used nitrocellulose-coated glass surface and the siliconized surface that we use suggests that the slowing of filaments is associated with the nitrocellulose surface (Figure 5). Notwithstanding this difference, we also found that the origin of the troponin and tropomyosin made a difference, since with sheep cardiac troponin/tropomyosin, we observed a greater decrease in filament velocity than we did with skeletal-muscle troponin/tropomyosin, when filaments were switched off at pCa 9.

The way in which troponin I and troponin C can switch filaments between motile and non-motile states is not clear. We have demonstrated that troponin binding to actin–tropomyosin is not co-operative, and it seems unlikely that switching between

motile and non-motile states is due to co-operative effects mediated by tropomyosin, since a number of laboratories have reported that calponin can 'switch off' actin-filament movement in the absence of tropomyosin [20,30,31]. The co-operativity therefore seems to reside in the interaction between the actin filament and the myosin bound to the siliconized surface. It is possible that filament movement needs a density of productive actin–myosin interactions above a critical threshold in order to overcome drag induced by the surface [32]. Troponin I, by promoting the steric blocking of 'strong' myosin-binding sites on actin, could act by reducing the density of productive actomyosin interactions below the critical concentration. This idea is supported by our observation that the reduction in concentration of myosin heads itself can induce a 'switch off' with similar characteristics to the troponin I effect. The absence of changes in filament velocity when filaments are switched off suggests that movement over HMM on the siliconized surface is largely friction-free, in contrast with movement on a nitrocellulose surface.

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