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Tropomyosin Inhibits the Rate of Actin Polymerization by Stabilizing Actin Filaments†

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ABSTRACT: Tropomyosin inhibition of the rate of spontaneous polymerization of actin is associated with binding of tropomyosin to actin filaments. Rate constants determined by using a direct electron microscopic assay of elongation showed that $\alpha\alpha$- and $\alpha\beta$-tropomyosin have a small or no effect on the rate of elongation at either end of the filaments. The most likely explanation for the inhibition of the rate of polymerization of actin in bulk samples is that tropomyosin reduces the number of filament ends by mechanical stabilization of the filaments.

The dynamics of actin polymerization and filament organization are affected by different types of actin binding proteins (Korn, 1982; Frieden, 1985; Pollard & Cooper, 1986). Tropomyosin interests us because it binds along the length of actin filaments and might have a role in length determination of thin filaments. Its role in the regulation of striated muscle contraction is well established (Leavis & Gergely, 1984; El Saleh et al., 1986). Though tropomyosin is present in most animal cells, the isoforms are different, and the functional significance of various isoforms is unknown (Côté, 1983).

Tropomyosin inhibits the rate of actin polymerization without altering the critical concentration (Pragay & Gergely, 1968; Wegner, 1982a; Hitchcock-DeGregori & Maris, 1983; Lal & Korn, 1986). Tropomyosin could affect the number of filaments (Wegner, 1982b; Wegner & Savko, 1982) and/or the rate at which each filament elongates. Wegner (1982b) reported that the kinetics of inhibition of polymerization could be explained by stabilization of actin against fragmentation, but in the absence of direct determination of the number of filament ends, he could not rule out an effect on elongation. Lal and Korn (1986) also reported inhibition of polymerization by tropomyosin but suggested that reduced rates of association and dissociation of subunits from the ends of filaments contributed to the overall effect.

We carried out similar experiments showing that inhibition of polymerization was associated with binding of tropomyosin to F-actin (Hitchcock-DeGregori & Maris, 1983). Since the inhibition was not overcome by addition of F-actin nuclei, we suggested that tropomyosin may inhibit elongation as well as

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fragmentation of filaments. We did not publish the results in full at the time because we were unable to measure the rates of association and dissociation at the filament ends directly and because of the publication of Wegner's paper after we had submitted our work in abstract form. Recently, an improved method for direct measurement of association and dissociation rates has been developed (Pollard, 1986). Using this method, we have shown that tropomyosin has no major effect on association or dissociation of monomers from filaments. We conclude that inhibition of the rate of polymerization by tropomyosin is due to the stabilization of actin filaments to fragmentation as a result of its binding to the actin filament.

Materials and Methods

Preparation of Proteins. Actin was purified from acetone powder of back and leg muscles of New Zealand white rabbits according to Hitchcock et al. (1982) or Spudich and Watt (1971). Following depolymerization, monomeric actin was separated from oligomers and minor contaminants by gel filtration on Sephadex G100 or G150 (MacLean-Fletcher & Pollard, 1980). G-Actin was stored in 5 mM Tris-HCl, pH 8.0, or 2 mM imidazole, pH 7.5, 0.5 mM DTT, 0.1 mM $CaCl_2$, 0.1 or 0.2 mM ATP, and 0.1 or 0.5 mM NaN_3 at 4 °C and used within a week.

Tropomyosin was prepared from rabbit skeletal muscle according to Hitchcock-DeGregori et al. (1985). Briefly, tropomyosin from the isoelectric precipitate obtained in preparation of troponin (Hitchcock et al., 1981) was purified by ammonium sulfate fractionation and hydroxyapatite chromatography (Eisenberg & Keilley, 1974) to separate $\alpha\alpha$- and $\alpha\beta$-tropomyosin. $\alpha\beta$-Tropomyosin was used for most experiments though comparable results were obtained with chicken $\alpha\alpha$-tropomyosin.

All operations were at 4 °C with the exception of actin polymerization which was at room temperature.

Polymerization Measurements. Viscosity measurements were carried out at 25 °C using Cannon-Ubbelholde semimicro viscometers, size 75, in which buffer has an outflow time of about 70 s. Light-scattering measurements were made at 25 °C in a Perkin-Elmer 650–10S fluorescence spectrophotometer with excitation and emission set at 400 nm. G-Actin was passed through a Millipore filter prior to initiation of polymerization to reduce interference by dust.

Polymerization was initiated by addition of an appropriate volume of a 20× polymerization buffer; the sample was vortexed gently and immediately transferred to a viscometer or cuvette.

Binding Measurements. Fully polymerized samples that had been used for viscosity or light-scattering measurements (1 mL) were sedimented at 20 °C in a Beckman ultracentrifuge for 3 h at 35 000 rpm in a type 50 rotor. The pellet was analyzed on 12% SDS–polyacrylamide gels according to Laemmli (1970).

Measurement of Elongation Rates by Electron Microscopy. The assays were carried out at room temperature as described by Pollard (1986) in 50 mM KCl, 2 mM $MgCl_2$, 1 mM EGTA, 0.1 mM ATP, 0.25 mM DTT, 50 μM $CaCl_2$, and 10 mM imidazole, pH 7.0, using freshly prepared *Limulus* acrosomal processes as nuclei. The nuclei were mixed 1:1 with actin to start the reactions. Two time points were taken for each actin concentration, and the rates of elongation were calculated from the linear dependence of length on time. When plotted as a function of actin concentration, these rates fit straight lines with correlation coefficients >0.99. The slope, x intercept, and y intercept gave the association rate constant, the critical concentration, and dissociation rate constant.

Table I: Effect of Tropomyosin on the Rate of Actin Polymerization[a]

condition	$t_{1/2}$ (min) viscosity	$t_{1/2}$ (min) I
100 mM KCl, 2 mM $MgCl_2$	6.3	9.8
+TM	12.5	14.0
100 mM KCl	18.0	25% at 220 min
+TM	48.0	ND
2 mM $MgCl_2$	6.7	11.5
+TM	17.0	17.0
0.5 mM $MgCl_2$	40.5	ND
+TM	35.5	ND

[a] [Actin] = 5.7 μM, [tropomyosin] = 1.2 μM in buffer containing 5 mM Tris-HCl, pH 7.5, 0.1 mM $CaCl_2$, 0.1 mM ATP, 0.5 mM DTT, and 0.2 mM NaN_3, 25.0 °C. ND = not determined.

Table II: Elongation Rate Constants and Critical Concentration in the Presence of Tropomyosin Relative to Control[a]

	k^+	k^-	C_0
	Barbed End ($x \pm$ SD, n = 3)		
$\alpha\alpha$TM/control	0.98 ± 0.06	1.54 ± 0.22	1.43 ± 0.14
$\alpha\beta$TM/control	0.77 ± 0.06	2.03 ± 1.6	2.23 ± 1.53
	Pointed End (x, n = 2)		
$\alpha\alpha$TM/control	1.04	1.49	1.70
$\alpha\beta$TM/control	1.12	0.70	0.70

[a] The experimental conditions were described under Materials and Methods and in the legend to Figure 6. The control is actin without tropomyosin.

Results

We used light scattering and viscometry to confirm previous observations (Wegner, 1982a; Lal & Korn, 1986) that both $\alpha\alpha$- and $\alpha\beta$-tropomyosin inhibit the rate of both spontaneous (Figure 1; Table I) and nucleated (Figure 2) polymerization of actin, without affecting the critical concentration for polymerization (Figure 3). The time to reach half-maximal polymerization was greater by a factor of 2–3 in the presence of tropomyosin. The effect was more pronounced in the viscometric assay where spontaneous polymerization is faster than in static samples, presumably due to the creation of new filament ends by fragmentation caused by shearing (Figure 1).

The inhibition of the rate of polymerization by tropomyosin occurred in three different conditions in which tropomyosin binds tightly to actin filaments, but not in 0.5 mM $MgCl_2$ where binding is weak (Table I; Figure 4). In fact, in 0.5 mM $MgCl_2$, there was a small but reproducible increase in the rate of polymerization. This suggests that the effect of tropomyosin requires (as expected) its binding to the actin filaments.

The inhibition of spontaneous polymerization by tropomyosin depends on the concentration of tropomyosin and is maximal at a molar ratio of 1 tropomyosin to 7 actins, where all filaments formed would be saturated with tropomyosin (Figure 5). This provides further evidence that it is the tropomyosin bound to the filaments that influences the rate of polymerization.

By a direct electron microscopic assay of elongation using *Limulus* sperm acrosomal processes as morphologically identifiable nuclei, an excess of $\alpha\alpha$-tropomyosin had little or no effect on the rate of elongation at either end of the filaments (Figure 6; Table II). However, with the $\alpha\beta$ isoform, we found a small but reproducible decrease in the association rate constant at the barbed end (Figure 6; Table II). Within the error of the measurements, all the other rate constants were the same controls and in the presence of either $\alpha\alpha$- or $\alpha\beta$-tropomyosin (Table II).

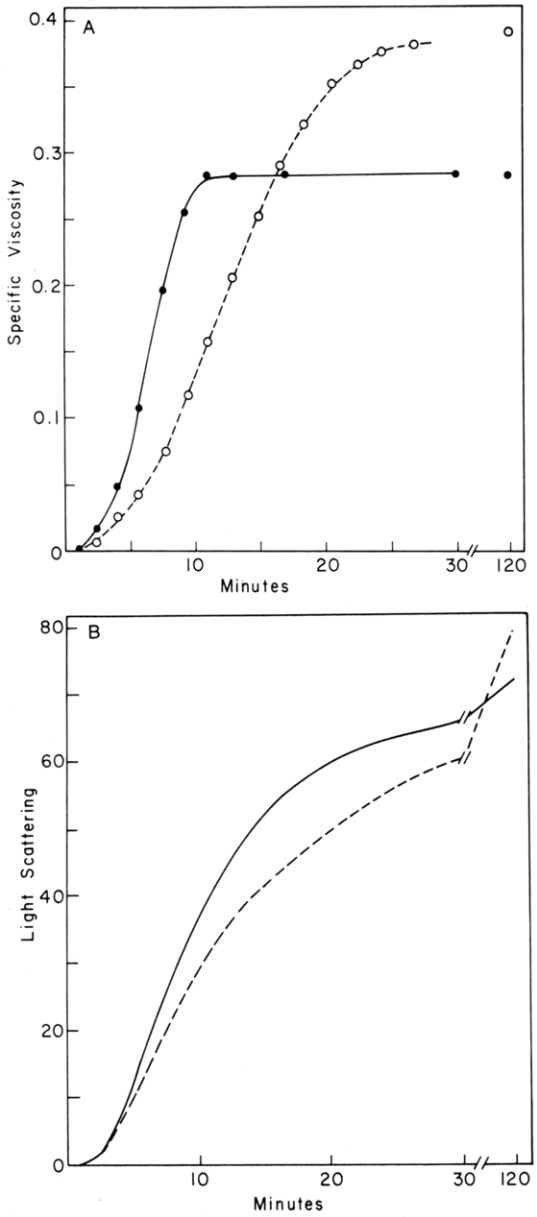

FIGURE 1: Time course of spontaneous polymerization of actin in the absence (—) and presence of tropomyosin (---). (A) Viscosity; (B) light scattering. Conditions: 5.7 μM actin monomers; 1.4 μM tropomyosin; 100 mM KCl, 2 mM $MgCl_2$, 5 mM Tris-HCl, pH 7.5, 0.1 mM $CaCl_2$, 0.1 mM ATP, 0.5 mM DTT, and 0.2 mM NaN_3, 25 °C. Polymerization was initiated by addition of 20× salt solution.

Discussion

The results of the current and previous investigations are in agreement that tropomyosin reduces the rate but not the extent of spontaneous polymerization of actin. The effect is caused by tropomyosin bound to the filaments. Since the reduced rate is observed for both spontaneous and nucleated polymerization, the effect is most likely on elongation rather than nucleation. Consequently, there are two main possibilities to consider for the mechanism. First, tropomyosin could inhibit both association and dissociation rates of monomer addition at one or both ends. This could be achieved by binding of tropomyosin along the length of the actin filament, thereby lowering the chemical potential of the actin polymer and thus lowering the association rate of monomer addition and dissociation (Hill, 1987). Since it is generally agreed that tropomyosin does not alter the critical concentration significantly, the hypothetical changes in association and dissociation rate constants would have to be exactly proportional in several buffers—a remarkable coincidence!

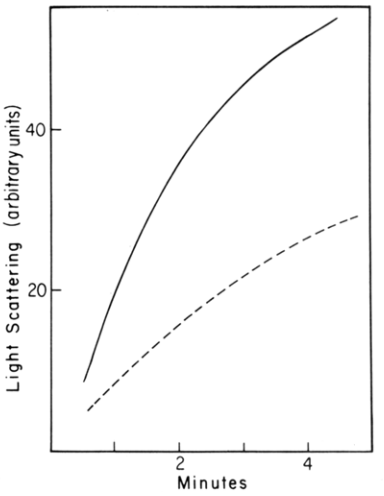

FIGURE 2: Time course of nucleated polymerization of actin in the absence (—) and presence of tropomyosin (---) followed by light scattering. The F-actin nuclei (0.6 μM) were added immediately after initiation of polymerization with salts at $t = 0$. Conditions: 6 μM actin; 2 μM tropomyosin; buffer as described in Figure 1.

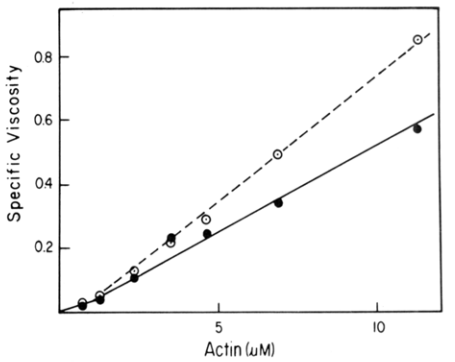

FIGURE 3: Measurement of actin critical concentration in the absence (—) and presence of tropomyosin (---). Conditions: 50 mM NaCl, 5 mM Tris-HCl, pH 7.5, 0.1 mM $CaCl_2$, 0.1 mM ATP, 0.5 mM DTT, and 0.2 mM NaN_3, 25 °C.

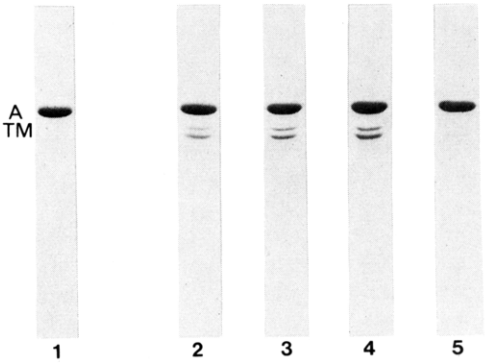

FIGURE 4: Evaluation of tropomyosin binding to actin filaments. Fully polymerized samples from experiments reported in Figure 1 and Table I were sedimented in the ultracentrifuge as described under Materials and Methods. The figure shows SDS–polyacrylamide gels of the pellets stained in Coomassie blue. Lane 1, actin alone; lanes 2–5, actin with tropomyosin: lane 2, 100 mM KCl and 2 mM $MgCl_2$; lane 3, 100 mM KCl; lane 4, 2 mM $MgCl_2$; lane 5, 0.5 mM $MgCl_2$.

Our results do not support this hypothesis. We find that tropomyosin has little or no effect on the rate constants for monomer addition and loss on individual filaments. The small decrease found in the association rate constant at the barbed end caused by $\alpha\beta$-tropomyosin could be explained by the mechanisms given above, but these effects cannot explain the

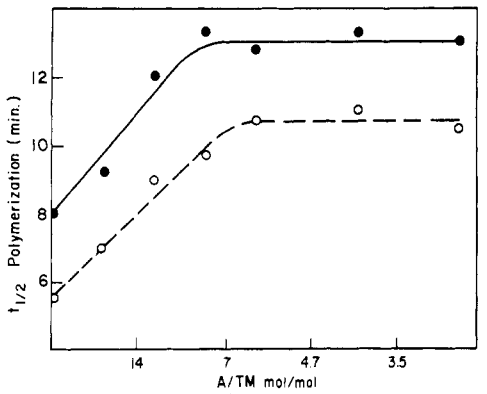

FIGURE 5: Inhibition of the rate of spontaneous actin polymerization measured by viscosity (---) and light scattering (—) as a function of the ratio of actin to tropomyosin. The concentrations were determined assuming a molecular weight of 42 000 for actin and 66 000 for tropomyosin. Conditions: 6 μM actin; variable tropomyosin concentration; buffer as described in Figure 1.

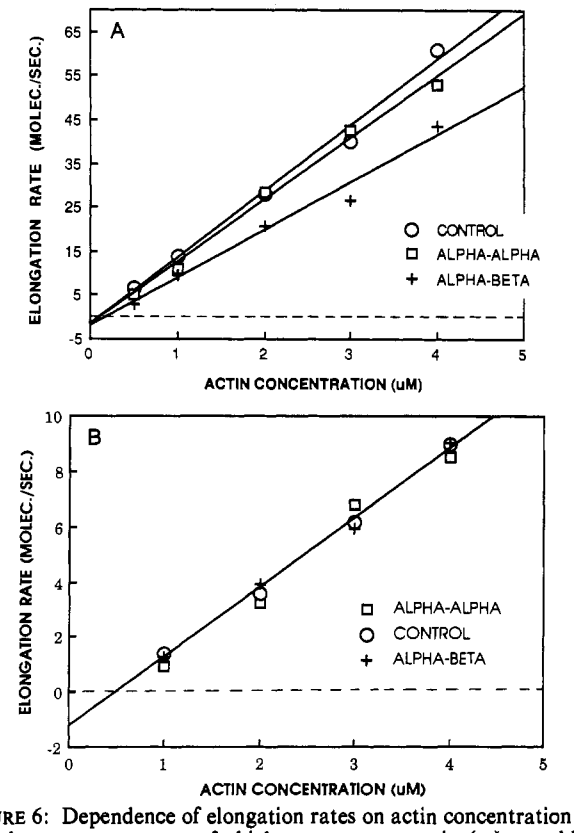

FIGURE 6: Dependence of elongation rates on actin concentration in the absence or presence of chicken $\alpha\alpha$-tropomyosin (□) or rabbit $\alpha\beta$-tropomyosin (+). Conditions: 2 μM tropomyosin; 50 mM KCl, 2 mM $MgCl_2$, 1 mM EGTA, 0.1 mM ATP, 0.25 mM DTT, 50 μM $CaCl_2$, and 10 mM imidazole, pH 7.0, 22 °C. In this experiment, the rate constants and critical concentration were the following. (A) (Barbed end) No TM: k^+, 15.1; k^-, 0.54; C_0, 0.05. $\alpha\alpha$TM: k^+, 13.5; k^-, 0.97; C_0, 0.08. $\alpha\beta$TM: k^+, 10.8; k^-, 2.1; C_0, 0.20. (B) (Pointed end) No TM: k^+, 1.6; k^-, 1.4; C_0, 0.66. $\alpha\alpha$TM: k^+, 1.8; k^-, 1.6; C_0, 0.65. $\alpha\beta$TM: k^+, 2.0; k^-, 0.20; C_0, 0.25. Units: k^+, μM^{-1} s^{-1}; k^-, s^{-1}; critical concentration, μM.

large change in the rate of polymerization in bulk samples.

A second mechanism is that the elongation reactions are unaffected but the number of ends available for growth might be influenced by the presence of tropomyosin. More specifically, the number of ends available for elongation may be lower in the presence of tropomyosin than in its absence. Our studies show that this second mechanism is dominant. Tropomyosin might promote end-to-end annealing of actin filaments, but we think that a reduction in filament number is explained most simply by the well-established ability of tropomyosin to mechanically stabilize filaments (Fujime & Ishiwata, 1971; Hitchcock et al., 1976; Bernstein & Bamburg, 1982; Bonder & Mooseker, 1983). Stabilization by tropomyosin should reduce the probability of filament fragmentation by thermal or mechanical forces. If this conclusion is correct, fragmentation of filaments in static samples of polymerizing actin may occur at a higher frequency than predicted by the most kinetic modeling (Zimmerle & Frieden, 1986).

Added in Proof

For another approach, please see the recently published paper of Wegner and Ruhnau (1988).

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