Nonpolymerizable tropomyosin, in which 11 residues have been quantitatively cleaved from the COOH terminus of muscle tropomyosin (TM) by enzymic digestion, does not bind to F-actin. Binding is restored in the presence of troponin (Tn) and absence of Ca$^{2+}$. The binding is stronger than for intact TM alone and shows residual cooperativity. In the presence of Ca$^{2+}$, the binding is at least 10-fold weaker and cooperativity is not observed. Tn-T alone is more effective than Tn-I alone in inducing nonpolymerizable TM binding. Tn-T plus Tn-I induce binding as effectively as whole Tn (without Ca$^{2+}$). In the absence of Ca$^{2+}$, Tn-T + Tn-C and Tn-I + Tn-C are more effective in promoting binding than in the presence of Ca$^{2+}$. These observations emphasize the importance of the head to tail overlap region of TM in the cooperative interactions of the thin filament assembly. The effects of Ca$^{2+}$ are largely understandable in terms of its known effects on the strength of interactions between Tn-I and TM + actin and between Tn-T and TM. The residual cooperativity observed in nonpolymerizable TM binding in the presence of Tn (without Ca$^{2+}$) may indicate that the T1 fragment region (residues 1-158) of Tn-T spans the head to tail overlap gap between the neighboring nonpolymerizable TM molecules. Alternatively, or in addition, the cooperativity may arise from conformational changes transmitted through actin from one nonpolymerizable TM-Tn binding site to others.

Recent evidence strongly supports the view that, while Tn-C and Tn-I are only moderately asymmetric (5, 6), Tn-T is a highly extended molecule which interacts with TM over an extensive region of the COOH-terminal half of its structure. Thus, the fragments CB1 and T1 of Tn-T (residues 1-151 and 1-158, respectively), which promote head to tail aggregation of TM and its fragments (7, 8), are believed to interact close to or at the COOH-terminal end of TM, possibly involving the head to tail overlap region (8-11). Fragment T2 of Tn-T (residues 159-259) has been shown to interact in the region of cysteine 190 of TM, a distance of some 14 nm from its COOH-terminal end (12-16). Available evidence (13, 14) strongly suggests that Tn-C and Tn-I are also located in the region of cysteine 190 of TM, a conclusion consistent with the observation that these components interact with Tn-T fragment T2 but not T1 (14, 17-19). Strong support for this asymmetric nature of Tn on the muscle thin filament has recently been obtained by an electron microscopic study of rotary shadowed Tn complex and Tn-T (20). The entire complex has both a globular (mostly Tn-I and Tn-C) and a rod-like domain with the tail (mostly Tn-T) having a length of 160 ± 35 Å.

An alternative view, that Tn is a more compact structure and interacts with TM over a more restricted region, is held by Nagano et al. (21), Ohtsuki (22), Nagano and Ohtsuki (23), and Ohtsuki and Nagano (24). However, their conclusions are based in part on predictive model building and neglect the accumulating chemical and structural evidence for the involvement of the COOH-terminal region of TM in its interaction with the T1 fragment region of Tn-T.

While the mechanism by which this assembly controls the contractile events is not fully understood, it is clear that the association of these thin filament proteins is strongly interdependent. Although TM interacts stoichiometrically (molar ratio 1:7) with F-actin under optimal salt conditions, nonpolymerizable TM, prepared by the removal of 11 amino acid residues from its COOH-terminal end, fails to bind (25). This is consistent with the observation that the binding curve of TM to F-actin is highly cooperative (26) and that the low binding constant for an isolated TM molecule (≈10$^{-7}$ M$^{-1}$) is dramatically increased by the interaction of contiguous TM molecules linked through their head to tail contacts (27, 28). Under conditions (low free [Mg$^{2+}$]) in which rabbit skeletal TM and platelet TM fail to bind to F-actin, binding can be induced by Tn-I and the S-1 fragment of myosin (29-31).

The interactions among the thin filament proteins are markedly dependent on Ca$^{2+}$ concentration. Thus, the binding of Ca$^{2+}$ to Tn-C leads to a strengthening of the interactions between Tn-C and both Tn-I and the T2 fragment region of Tn-T (17, 32-35), to a change in the interactions between
between Tn-I and TM-F-actin (37-39) and between the T2 fragment of Tn-T and TM is relatively independent of Ca\(^{2+}\) but may be affected by transmitted conformational changes induced by changes in the interaction of Tn-I and the fragment T2 region with TM in the region of cysteine 190 (11).

To provide further information on the structural and functional relationship of this complex system and, in particular, of the role of the head to tail overlap region of TM molecules, we have previously described the preparation of nonpolymerizable TM and reported its inability to bind to F-actin and the CB1 and T1 fragments (residues 1-151 and 1-158, respectively) of Tn-T (9, 25). In the present investigation, we report the effectiveness of Tn and of the Tn components, alone or in combination, in the presence and absence of Ca\(^{2+}\) for the induction of nonpolymerizable TM binding to F-actin.

**MATERIALS AND METHODS**

**Preparation of TM, Nonpolymerizable TM and Actin—**Rabbit skeletal muscle was obtained from a local slaughter house and is identical with that from rabbit skeletal muscle (40). Nonpolymerizable TM was prepared as previously described (25). For some experiments, the nonpolymerizable TM was further purified by treatment with F-actin, which binds intact TM but not nonpolymerizable TM, using the same conditions as described below for the actin binding experiments. The TM-F-actin complex was pelleted at 97,000 × g for 90 min. The nonpolymerizable TM in the supernatant was purified from actin by chromatography on CM32 using the same conditions as described previously for the fractionation of α- and β-TMs (41). No differences were observed between the two nonpolymerizable TM preparations in the experiments described in this paper. Actin was extracted and purified from acetone powders of rabbit skeletal muscle (42). The G-actin was stored at 4 °C and used within 2 weeks of preparation.

**Preparation of Tn and Its Components—**The Tn complex was prepared from rabbit skeletal muscle as described (43). The method of Hartshorne and Mueller (44) was modified to fractionate the Tn into Tn-C and a mixture of Tn-I and Tn-T. To 1 g of Tn complex dissolved in 1 liter of 0.05 M HCl, 10 mM β-mercaptoethanol at room temperature, 120 ml of 10% perchloric acid at room temperature were added slowly. After gentle stirring for 30 min, Tn-C was collected by centrifugation at room temperature at 15,700 × g for 15 min. The Tn-C pellet was dissolved in 5 mM β-mercaptoethanol, adjusted to pH 7.0, dialyzed exhaustively at 4 °C against 2 mM β-mercaptoethanol, and lyophilized. It was further purified on a DEAE-Sephadex A-25 column as described by Byers and Kay (45). Tn-I and Tn-T in the supernatant were collected by centrifugation, 10 min at 15,700 × g for 10 min. The Tn-I and Tn-T were separated on a CM-cellulose column as described by Wilkinson (46).

**RESULTS**

**Binding of Intact TM and Nonpolymerizable TM to F-actin—**The results of binding experiments in which the actin concentration was maintained at 7 μM and the TM or nonpolymerizable TM concentration was increased from 0 to 3 μM or higher are shown in Fig. 1A. The sigmoidal binding curve of TM with actin indicates a highly cooperative process. This is also demonstrated clearly in the curved Scatchard plot in Fig. 1B. The apparent constant for the binding of TM to actin, K, expressed as the reciprocal of the free TM at half-saturation of the actin filaments, was 1.7 × 10⁶ M⁻¹. These results are in good agreement with similar experiments previously carried out by Yang et al. (26).

When the interaction of nonpolymerizable TM with F-actin was examined under identical conditions, negligible binding was observed (Fig. 1A). This observation is consistent with the data of Wegner (27) and Walsh and Weggish (28) who from light scattering measurements calculated that the equilibrium constant for binding of an isolated TM molecule on F-actin was ~10³⁻. The binding was increased ~10³⁻.

**FIG. 1.** A, effects of Tn (±Ca\(^{2+}\)) on the binding of nonpolymerizable TM to F-actin. Binding of TM alone (Δ), nonpolymerizable TM alone (A), nonpolymerizable TM + Tn (—Ca\(^{2+}\)) (O), and nonpolymerizable TM + Tn (+Ca\(^{2+}\)) (●) to F-actin. In all experiments, the actin monomer concentration was 7 μM and the concentration of TM or nonpolymerizable TM (NPTM) was varied. When present, the concentration of Tn was 2 μM. The buffer conditions were described under “Materials and Methods.” B, Scatchard plot of data calculated directly from those of A. Symbols are the same. x is the moles of bound TM (or nonpolymerizable TM) per mol of actin monomer.
10-fold when the interaction of contiguous TM molecules linked head to tail was considered. Since nonpolymerizable TM lacks the capacity to form head to tail contacts (25), its interaction with actin is equivalent to that of a single isolated TM molecule and with the protein concentrations (1–10 μM) used in these experiments, negligible binding is expected. Since the concentration of Mg2+ has been shown to significantly affect the interaction of TM with actin (26, 27, 31), the Mg2+ concentration was varied from 2 to 8 mM under otherwise identical conditions. No effect on the binding of nonpolymerizable TM to actin was observed (data not shown), indicating that the enhancing effects of Mg2+ in promoting binding are not adequate to overcome the absence of cooperativity resulting from the lack of head to tail interaction in nonpolymerizable TM.

**Effect of Tn (±Ca2+) on Binding of Nonpolymerizable TM to F-actin**—As shown in Fig. 1A, the Tn complex fully restored the binding of nonpolymerizable TM to F-actin in the absence of Ca2+. Comparison of the binding curves for nonpolymerizable TM + Tn and TM to F-actin shows that the binding of nonpolymerizable TM + Tn is stronger but less cooperative than the binding of untreated TM to F-actin with apparent K values of 2.9 (±0.1) × 10^6 M^{-1} and 1.7 (±0.1) × 10^6 M^{-1}, respectively. The presence of residual cooperativity in the binding of nonpolymerizable TM + Tn to actin is confirmed by the Scatchard plot of the data in Fig. 1B which shows a curved but less convex plot than for the binding of untreated TM to F-actin and with a maximum shifted to lower values of ν.

In the presence of Ca2+, the Tn complex only partially restored the binding of nonpolymerizable TM to F-actin. Within the range of nonpolymerizable TM concentrations used in the present experiments (up to 3.6 μM), saturation of the F-actin filaments was not observed. The equilibrium constant can therefore only be estimated at 0.2 × 10^6 M^{-1}, a value at least 10-fold lower than for the binding of nonpolymerizable TM + Tn in the absence of Ca2+. In the present experiments, no evidence for cooperativity in the binding of nonpolymerizable TM + Tn (+Ca2+) to F-actin was obtained (see Scatchard plot of Fig. 1B).

To investigate further the effect of the Tn complex on the binding of nonpolymerizable TM to F-actin, the actin and nonpolymerizable TM concentrations were kept constant at 7 and 2 μM, respectively, while the concentration of Tn was varied from 0 to 7 μM. As shown in Fig. 2, Tn (+Ca2+) restored the stoichiometric binding of nonpolymerizable TM to actin when the molar ratio of Tn to actin was in excess of 0.3. On the other hand, Tn (+Ca2+) only partially restored the binding even at higher Tn to actin ratios.

**Effects of Tn-T, Tn-I, and Tn-T + Tn-I on Binding of Nonpolymerizable TM to F-actin**—As shown in Fig. 3, Tn-T alone can substantially enhance the binding of nonpolymerizable TM to actin, reaching a value of 85% saturation at a molar ratio of Tn-T to actin of 0.8. Tn-I, on the other hand, increased the affinity of nonpolymerizable TM and actin to a lesser extent, reaching a level of about 50% saturation at high molar ratios of Tn-I to actin. Tn-C alone had no effects on the binding of nonpolymerizable TM to actin (data not shown).

**Fig. 2.** Induction of binding of nonpolymerizable TM to F-actin by Tn in the absence and presence of Ca2+. Actin monomer concentration was 7 μM and the Tn concentration was varied. Nonpolymerizable TM (NPTM) concentration was 2 μM. Buffer conditions were as in Fig. 1. Experimental points are the average of at least two determinations.

**Fig. 3.** Effects of Tn-T, Tn-I, and Tn-T + Tn-I on the binding of nonpolymerizable TM to F-actin. Actin monomer and nonpolymerizable TM (NPTM) concentrations were 7 and 2 μM, respectively, while the concentrations of the Tn components were varied. The molar ratio of Tn-T to Tn-I was 1:1. Buffer conditions were as in Fig. 1. Experimental points are the average of at least two determinations.

**Fig. 4.** Effects of Tn-T + Tn-C (±Ca2+) and of Tn-I + Tn-C (±Ca2+) on the binding of nonpolymerizable TM to F-actin. Actin monomer and nonpolymerizable TM (NPTM) concentrations were 7 and 2 μM, respectively, while the concentrations of the Tn components were varied. The molar ratios of Tn-T to Tn-C and of Tn-I to Tn-C were 1:1. Buffer conditions were as in Fig. 1.
shown). When the effects of Tn-T + Tn-I were tested, full induction of nonpolymerizable TM binding to actin was observed and the binding curve was similar to that observed with whole Tn (-Ca\(^{2+}\)); compare Figs. 2 and 3. These results show that the effects of Tn (-Ca\(^{2+}\)) on the binding of nonpolymerizable TM to actin are mediated largely through Tn-T but that Tn-I also participates in this phenomenon and potentiates the effect of Tn-T. The presence of Tn-C in the absence of Ca\(^{2+}\) would appear to have little effect.

Effect of Tn-T + Tn-C (+Ca\(^{2+}\)) and Tn-I + Tn-C (+Ca\(^{2+}\)) on the Binding of Nonpolymerizable TM to Actin—As shown in Fig. 4, Tn-C (-Ca\(^{2+}\)) has little effect on the binding induced by Tn-T of nonpolymerizable TM to actin (compare Figs. 3 and 4). In the presence of Ca\(^{2+}\), however, Tn-C significantly reduces the effect of Tn-T. The presence of Tn-C on the binding induced by Tn-I significantly reduces the latter's effect (compare Figs. 3 and 4) to a level where an effect of Ca\(^{2+}\) on the induction was not experimentally significant.

**DISCUSSION**

In the present work, a preparation of nonpolymerizable TM has been employed which differs from intact TM in that residues 274–284 have been quantitatively removed by treatment with carboxypeptidase A (9, 25). Previous studies (25) have demonstrated that this preparation has completely lost its ability to polymerize in a head to tail manner and has a $M_\text{r}$ of 66,000 in solution even at low ionic strength. It retains, however, its coiled coil structure with only minimal changes in its stability properties (25). Interestingly, this preparation of nonpolymerizable TM no longer binds to F-actin under conditions known to be optimal for the binding of intact TM, demonstrating the importance of the head to tail interaction of contiguous TM molecules in the cooperative interaction of TM with F-actin. These observations are in excellent agreement with the studies of Wegner (27) and Walsh and Wegner (28) which indicate that the equilibrium constant for the binding of TM to singly contiguous sites on the actin filament is 600–1200 times greater, depending on the Mg\(^{2+}\) concentration, than that for the binding of TM to isolated sites. In addition, it has been observed that platelet TM, which displays a significantly weaker tendency to aggregate in a head to tail manner, also binds more weakly to F-actin relative to intact skeletal TM under similar experimental conditions. In the case of the platelet TM, however, the binding to F-actin can be induced to stoichiometric levels by increasing the Mg\(^{2+}\) concentration to 8–10 mM (31). In the present work with nonpolymerizable TM, this was not observed. We conclude that the effects of increasing Mg\(^{2+}\) concentration in inducing the binding of intact skeletal TM and platelet TM to F-actin are inadequate to promote such binding in the complete absence of head to tail polymerization, as is the case with nonpolymerizable TM.

While nonpolymerizable TM by itself fails to bind to F-actin, the present studies demonstrate that the addition of Tn complex to the system in the absence of Ca\(^{2+}\) ions restores stoichiometric binding. The apparent constant for this binding is in fact almost 2-fold greater than for intact muscle TM alone under the same conditions. Interestingly, the binding curve and Scatchard plot demonstrate a significant degree of residual cooperativity in this interaction. In the presence of Ca\(^{2+}\), the Tn complex is much less effective in inducing nonpolymerizable TM binding to actin. When the effect of Tn-C, which reportedly does not bind to F-actin, is included, the apparent binding constant estimated to be at least 10-fold lower than in the absence of Ca\(^{2+}\). No cooperativity in this binding could be detected in the present experiments.

To further investigate the molecular basis of these observations with whole Tn, we examined the effects of individual Tn components either alone or in combination for their ability to induce nonpolymerizable TM binding to F-actin. Tn-T was most effective, while Tn-I produced a significant but lesser induction of binding (see Fig. 3). The addition of both Tn-T and Tn-I induced nonpolymerizable TM binding to a level equivalent to that observed with whole Tn in the absence of Ca\(^{2+}\). Thus, Tn-C would appear to have little effect on the binding when Ca\(^{2+}\) is absent. While the induction of binding by Tn-I is understandable since it is known to interact with both TM and actin, that of Tn-T is less obvious since it interacts only with TM and reportedly not with actin (38, 39). The interaction of Tn-T with intact TM is now known to involve two regions on each of these two proteins. Thus, fragment T2 (residues 159–259) of Tn-T binds to TM in the region of cysteine 190, about one-third of the molecular distance from its COOH-terminal end (12–16). With Ca\(^{2+}\), this interaction is disrupted in the presence of Tn-I and Tn-C to which this fragment also binds (10, 11). On the other hand, fragment T1 (residues 1–158) of Tn-T binds close to or at the COOH-terminal end of the TM molecule, possibly involving the head to tail overlap region (9). This interaction is insensitive to the Ca\(^{2+}\) concentration of Tn-C, although it may be affected by conformational changes transmitted from the fragment T2 binding region through the TM molecule (11). In the case of nonpolymerizable TM, this interaction with the T1 (or CB1) region (residues 1–158 (or 1–151)) has been shown to be significantly weakened but perhaps not eliminated (9, 10). Thus, the significant effects of Tn-T on the induction of binding of nonpolymerizable TM to actin may be explicable in terms of a bridging of the gap between adjacent nonpolymerizable TM molecules created by the removal of the COOH-terminal T1 residues to produce nonpolymerizable TM. Such an interpretation would be consistent with the residual cooperativity of binding of nonpolymerizable TM observed in the presence of whole Tn in the absence of Ca\(^{2+}\) (see Fig. 1, A and B).

The effects of the addition of Ca\(^{2+}\) in reducing the induction of binding of nonpolymerizable TM by Tn-T + Tn-C, by Tn-I + Tn-C, and by whole Tn are not unexpected, since the interactions between Tn-I and F-actin + TM and between the T2 fragment region of Tn-T and TM have been shown to be weakened by Ca\(^{2+}\) in a system also containing Tn-C (11). In the case of the strength of interaction of fragment T1 of Tn-T with the head to tail overlap region of TM, no direct effect of Ca\(^{2+}\) is anticipated, nor has one been observed since this segment of Tn-T does not interact with Tn-C. However, we have shown previously (11) that the binding of Tn-I to TM leads to an increased affinity of interaction between the Tn-T fragment T1 and TM, presumably through a conformational change transmitted through TM from the region of its Cys-190 residue to its head to tail overlap. If one accepts the view that in the case of nonpolymerizable TM, the T1 region of Tn-T may bridge the gap between adjacent nonpolymerizable TM molecules, this interaction could thus be further indirectly weakened by the binding of Ca\(^{2+}\) to Tn-C. This could lead to a reduction in the binding of nonpolymerizable TM induced by Tn in the presence of Ca\(^{2+}\) and to a loss of cooperativity in that binding, consistent with the observations in the present study. Alternatively, or in addition, Tn in the absence of Ca\(^{2+}\) may induce cooperative binding of nonpolymerizable TM by transmission of conformational changes through the F-actin structure. In the case of Tn-T alone, which reportedly does not bind to F-actin, such induced binding could only occur by a series of conformational changes transmitted from nonpolymerizable TM.
through actin. At the present level of understanding of the system, it is not possible to distinguish among these possibilities nor to assess their contributions to the observed effects.

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REFERENCES

1. Weber, A., and Murray, J. M. (1973) Physiol. Rev. 53, 612-673
2. Mannherz, H. G., and Goody, R. S. (1976) Annu. Rev. Biochem. 45, 427-465
3. Smillie, L. B. (1979) Trends Biochem. Sci. 4, 151-155
4. McCubbin, W. D., and Kay, C. M. (1980) Acc. Chem. Res. 13, 185-192
5. Byers, D. M., and Kay, C. M. (1982) Biochemistry 21, 229-233
6. Byers, D. M., and Kay, C. M. (1983) J. Biol. Chem. 258, 2951-2964
7. Jackson, P., Amphlett, G. W., and Perry, S. V. (1975) Biochem. J. 151, 85-97
8. Pato, M. D., Mak, A. S., and Smillie, L. B. (1981) J. Biol. Chem. 256, 7137-7140
9. Pellet, I., and De Gregori, S. E. (1982) J. Biol. Chem. 257, 7372-7380
10. Margossian, S. S., and Cohen, C. (1973) J. Mol. Biol. 81, 409-413
11. Poter, J. D., and Gergely, J. (1974) Biochemistry 13, 2697-2703
12. Hitchcock, S. E., Huxley, H. E., and Szent-Gyorgyi, A. G. (1973) J. Mol. Biol. 80, 825-836
13. Lewis, W. G., and Smillie, L. B. (1980) J. Biol. Chem. 255, 8654-8659
14. Stone, D., and Smillie, L. B. (1978) J. Biol. Chem. 253, 1137-1148
15. Spudich, J. A., and Watt, S. (1971) J. Biol. Chem. 246, 4866-4871
16. Ebashi, S., Wakabayashi, T., and Ebashi, F. (1971) J. Biol. Chem. (Tokyo) 69, 441-445
17. Hartshorne, D. J., and Mueller, H. (1968) Biochem. Biophys. Res. Commun. 31, 647-653
18. Byers, D. M., and Kay, C. M. (1982) Biochemistry 21, 229-233
19. Wilkinson, J. M. (1974) Biochim. Biophys. Acta 359, 379-388
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