Separation and Characterization of the Two Functional Regions of Troponin Involved in Muscle Thin Filament Regulation†

S. Schaertl,‡,§ S. S. Lehrer,*,|| and M. A. Geeves*,‡

Max Planck Institute für Molekulare Physiologie, Postfach 102664, 44026 Dortmund, Germany, Muscle Research Laboratories, Boston Biomedical Research Institute, 20 Staniford Street, Boston, Massachusetts 02114, and Department of Neurology, Harvard Medical School, Boston, Massachusetts 02115

Received August 30, 1995; Revised Manuscript Received October 24, 1995⊗

ABSTRACT: Mild proteolytic cleavage of the troponin complex yields TnT_1, the N-terminal fragment of troponin T, and TnT_2IC, a complex of the C-terminal fragment of troponin T (TnT_2) with troponin I (TnI) and troponin C (TnC) [Morris, E. P., & Lehrer, S. S. (1984) *Biochemistry 23*, 2214−2220]. Both TnT_1 and TnT_2IC bind tightly to the tropomyosin·actin (Tm·actin) thin filament and influence the interaction of myosin subfragment 1 (S1) with Tm·actin. TnT_1 does not affect the rate of S1 binding to Tm·actin but does increase the cooperativity with which S1 "turns on" Tm·actin, monitored by the excimer fluorescence of a pyrene label attached to Cys 190 of Tm [Geeves, M. A., & Lehrer, S. S. (1994) *Biophys. J. 67*, 273−282]. The apparent cooperative unit size of Tm·actin is increased from 6 to 9 by TnT_1 and to 12 by whole troponin. In contrast, TnT_2IC has no effect on the cooperativity of Tm·actin but does make the apparent S1-binding rate constant, k_{app}, Ca^{2+}-sensitive; i.e., in the absence of Ca^{2+}, k_{app} is reduced 2−3-fold by both TnT_2IC and whole troponin. Thus, the N- and C-terminal regions of TnT appear to act independently in modulating effects of S1 binding to the Tm·actin thin filament that are important in regulation.

The interaction between actin (A) and myosin (M) or its proteolytic fragment, S1, in striated muscle is regulated by Ca^{2+}-induced changes in the state of the muscle thin filament composed of actin, tropomyosin (Tm), and troponin (Tn) [for a review, see Lehrer (1994)]. The influence of Tn/Tm on the interaction between actin and myosin can be interpreted in terms of two Ca^{2+}-sensitive equilibria of the thin filament, blocked−closed and closed−open, which affect the actin-activated ATPase activity (Lehrer, 1994; McKillop & Geeves, 1993):

		K_B		K_T	
thin filament state:	blocked	↔	closed	↔	open
activity state:	off		off		on

In the blocked state, the binding of S1 to form weakly bound actin−S1 complexes is inhibited. In the closed state, the formation of a weakly bound actin−S1 complex (A-type) is not inhibited, but isomerization of this complex to the strongly bound R-type is inhibited. Only in the open state can strongly bound R-type complexes be formed, and these are required for stimulation of the ATPase of the bound S1 or contraction of a muscle fiber. Thus the blocked and closed states are both "off" and the open state is "on" with respect to actin-activated myosin ATPase activity (Lehrer, 1994). In the absence of Tn and Tm, the actin filament is always in the open (on) state (Table 1). Addition of Tm produces the closed−open equilibrium, and, in the additional presence of Tn, the closed−open equilibrium becomes dependent on the Ca^{2+} concentration. In the presence of Tn the blocked state predominates at low Ca^{2+}, but the blocked state is lost at high Ca^{2+} (Head et al., 1995; McKillop & Geeves, 1993). Both actin·Tm and actin·Tm·Tn filaments are predominantly in the off state (closed + blocked) in the absence of S1 (Geeves & Halsall, 1987; Lehrer & Morris, 1982; Williams et al., 1988). The binding of S1 to actin to form the R-state cooperatively shifts the thin filament to the open state (Geeves & Halsall, 1987; Greene & Eisenberg, 1980). Ca^{2+} is a modulator of this cooperative transition (Lehrer, 1994). The number of actin subunits that are switched to the open state by the binding of a single S1 is the apparent cooperative unit of the thin filament, n (Geeves & Lehrer, 1994).

Table 1: Fractional Occupancy of the Three States of the Thin Filament

thin filament	blocked	closed	open
actin			1.0
actin·Tm		0.8	0.2
actin Tm·Tn + Ca^{2+}		0.8	0.2
actin·Tm·Tn − Ca^{2+}	0.7	0.25	0.05
any filament + S1[a]			1.0

[a] Strongly bound R-type complexes.

The excimer fluorescence of Tm, specifically labeled at Cys 190 with pyrene iodoacetamide (Tm*), monitors the closed-to-open transition of the filament (Ishii & Lehrer, 1990). With the use of Tm*, Geeves and Lehrer (1994) showed that n = 5−6 for actin·Tm filaments and 10−12 for actin·Tm·Tn filaments. Thus, addition of Tn to the Tm·actin filament has two effects. It introduces Ca^{2+} dependence to the two thin filament equilibria and increases the size of the cooperative unit.

Tn consists of three interacting proteins, TnC (the Ca^{2+} binding component), TnI (the inhibitory component), and

† Supported by a NATO Grant and by NIH Grant HL22461 (S.S.L.).
* Corresponding authors.
‡ Max Planck Institute für Molekulare Physiologie.
§ Current address: Max Planck Institute für Biophysical Chemistry, Department of Molecular Genetics, Göttingen, Germany.
|| Boston Biomedical Research Institute and Harvard Medical School.
⊗ Abstract published in *Advance ACS Abstracts,* December 1, 1995.

0006-2960/95/0434-15890$09.00/0 © 1995 American Chemical Society

TnT (the Tm-binding component that also is linked to TnI and TnC) (Leavis & Gergely, 1984). Previous studies have obtained evidence for an elongated Tn (Flicker et al., 1982; Ohtsuki, 1979) containing TnT_2, the C-terminal part of TnT which interacts with TnC−TnI, and TnT_1, the N-terminal part of TnT (Heeley et al., 1987). When Tm is bound to actin, TnT_1 appears to overlap a few N-terminal residues of the neighboring Tm molecule (Brisson et al., 1986), whereas the TnC/TnI/TnT_2 region appears to interact with the middle of Tm (Morris & Lehrer, 1984; Pearlstone & Smillie, 1982). Proteolytic cleavage of Tn under mild conditions produces TnT_1 (residues 1−158) and TnT_2IC, the complex of TnI and TnC with TnT_2 (residues 159−259) (Morris & Lehrer, 1984).

Here we report the effects of TnT_2IC and TnT_1 separately on the kinetics of S1 binding to thin filaments and the kinetics of the thin filament state change. We demonstrate that TnT_1 increases the apparent size of the cooperative unit of the actin·Tm filament whereas TnT_2IC, introduces Ca^{2+} sensitivity to the thin filament, but has little influence on the apparent size of the cooperative unit. Thus, we have succeeded in separating the functional properties of Tn, which involve two parts of TnT behaving in a relatively independent manner.

MATERIALS AND METHODS

Myosin subfragment 1 (S1) was prepared by chymotryptic digestion of rabbit muscle myosin as previously described (Weeds & Pope, 1977) and stored by freeze-drying in the presence of 1 mg of sucrose per mg of protein. Stock solutions were prepared by dialyzing out the sucrose before use. Actin was freshly prepared from an acetone powder of rabbit muscle and purified by two cycles of polymerization depolymerization (Lehrer & Kerwar, 1972; Spudich & Watt, 1971).

Tm and Tn were prepared from rabbit skeletal muscle, and Tm was labeled with pyrene iodoacetamide as described earlier (Ishii & Lehrer, 1990). TnT_1 was prepared by chymotryptic digestion of TnT and TnT_2IC by digestion of whole Tn as described by Morris and Lehrer (1984).

Light scattering (LS) and fluorescence (Fl) kinetic signals were obtained using a Hi-Tech Scientific SF-51 or SF-61 stopped-flow spectrophotometer equipped with a 100 W Xe/Hg lamp and monochromator. Excimer fluorescence, excited at 365 nm, was detected after passing through a GG 455 nm cut-on filter to remove light scattering and monomer fluorescence. Light scattering was observed at 90° to the incident light (405 nm) without filters. A total of 500 12-bit data points were collected using a DAS 50 A to D converter in a Hewlet Packard 486 computer with Hi-Tech software. Three to six traces of LS and Fl data were collected with the same diluted stock solution, averaged, and fitted to exponentials using a nonlinear least-squares fitting routine. Concentrations always refer to the concentrations of the reactants after mixing in the stopped flow spectrophotometer. The experimental buffer used throughout was 0.14 M KCl, 5 mM $MgCl_2$, and 20 mM MOPS pH 7.0, 20 °C.

RESULTS AND DISCUSSION

Effects of TnT_2IC and Whole Tn on Kinetics of Binding S1 to Actin·Tm. The rates of S1 binding to actin·Tm*·T_2IC and actin·Tm*·Tn were compared by monitoring light scattering changes in the stopped flow apparatus. The rapid

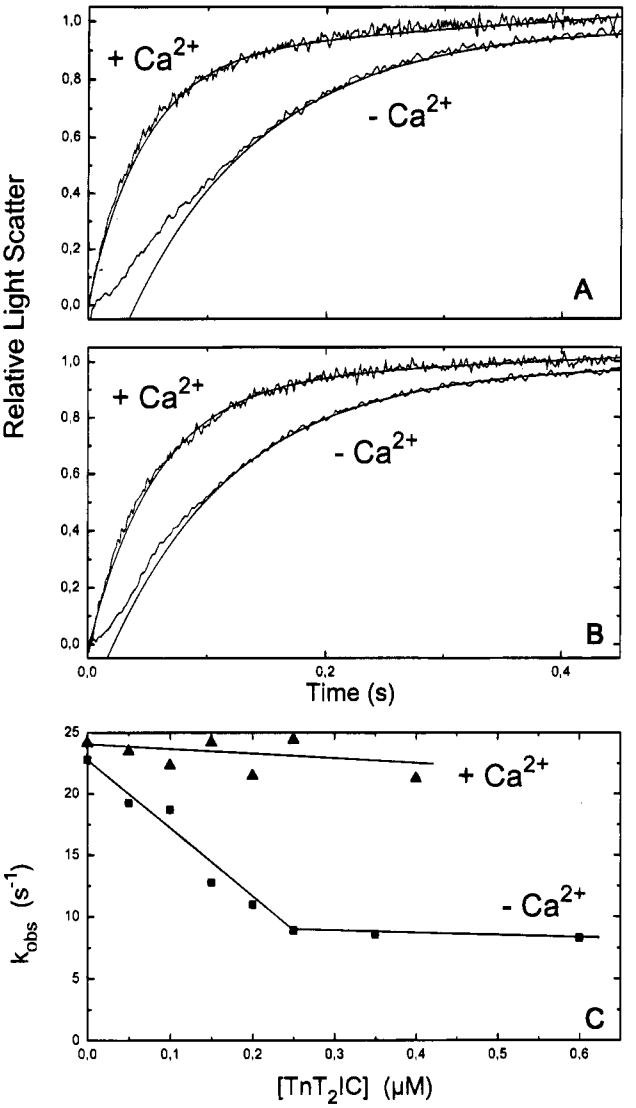

FIGURE 1: Influence of TnT_2IC and Tn on the kinetics of binding of S1 to actin·Tm* in the presence and absence of Ca^{2+}. (A) Tn; (B) TnT_2IC. The observed LS signals are shown with a single-exponential least-squares fit to the whole curve (+Ca^{2+}, 100 μM $CaCl_2$) or to the last 50% of the curve (−Ca^{2+}, 2 mM EGTA). (C) Titration of the observed rate constants against TnT_2IC concentration in the absence and presence of Ca^{2+}. Lines were drawn by eye. Experimental conditions: 10 μM S1, 1 μM actin, 0.15 μM Tm*, and (A) 0.2 μM Tn or (B) 0.25 μM TnT_2IC.

mixing of excess S1 (10 μM) with actin·Tm*·Tn filaments (1 μM actin) in the presence of Ca^{2+} produced an exponential increase in light scattering (Figure 1A) which appears identical to the reaction in the absence of Tm*·Tn. In the absence of Ca^{2+} the light scattering changed in two stages, an initial slow change, which then accelerated as the first few S1s to bind, switched "on" the thin filament leading to faster binding of the later S1 molecules (McKillop & Geeves, 1993; Trybus & Taylor, 1980). This switching on process involves a cooperative loss of the blocked state which cannot bind S1. With the TnT_2IC complex in place of whole Tn, the results of the S1 binding measurements were very similar to those of whole Tn (Figure 1B). Thus a slowing of the initial rate of binding of S1 to the filament in the absence of Ca^{2+} was observed which indicates the presence of the blocked state. The similarity of the binding reactions for whole Tn and TnT_2IC suggests that the calcium sensitive formation of the blocked state only requires the TnT_2 part

of TnT in a complex with TnI and TnC; i.e., the TnT_1 part of TnT is not required for either Ca^{2+} sensitivity or the blocked state. The small difference between the acceleration phases when comparing whole Tn and TnT_2IC may be accounted for by small changes in the cooperativity of the filament.

The effect of TnT_2IC concentration on the observed rate constant for S1 binding to actin·Tm filaments is shown in Figure 1C. In the presence of Ca^{2+}, TnT_2IC has little effect on k_{obs} yet this not due to the lack of TnT_2IC binding since fluorescence titrations using pyrene-labeled Tm (Tm*) indicated that TnT_2IC binds tightly to actin·Tm* both in the absence and presence of Ca^{2+} (data not shown). In the absence of Ca^{2+} a lag phase became apparent as the TnT_2IC concentration was increased. The k_{obs}, obtained by fitting an exponential to the last 50% of the scattering change, i.e., after any lag phase was complete, was reduced linearly as the TnT_2IC concentration was increased (Figure 1C). The effect saturated when 1 TnT_2IC was present per 4−5 actins or 1.4 TnT_2IC per 7 actins. The slight excess over 1 per 7 required to produce saturation probably reflects the presence of some inactive protein. Thus the effect of TnT_2IC on the S1 binding reaction is very similar to that of whole Tn; on binding stoichiometric amounts of either Tn or TnT_2IC, the S1 binding rate to actin·Tm is decreased only in the absence of Ca^{2+}.

Our previous work showed that the light scattering (LS) and pyrene excimer fluorescence (Fl) changes produced by S1 binding to actin·Tm* were single exponentials, and the observed rate constants for both signal changes k_{LS} and k_{Fl} were linearly dependent on [S1] (for [S1] ≫ [actin]). Thus both the binding and changes in state are limited by the rate of binding of S1 to actin. For actin·Tm* filaments, k_{Fl}/k_{LS} = 5−6, and for actin·Tm*·Tn filaments the ratio was 10−12. Each S1 that binds to actin produces a light scattering change and $k_{LS} = k_{on}[S1]$, where k_{on} is the second order rate constant for the binding reaction. If the first S1 binding to any of the actin sites within the cooperative unit produces both the thin filament state change and the Tm* fluorescence change, the fluorescence signal change is proportional to the concentration of actin units affected and $k_{Fl} = nk_{on}[S1]$, where n is the number of binding sites within the cooperative unit. Thus, $k_{Fl} = nk_{LS}$, and the ratio, k_{Fl}/k_{LS}, is a direct estimate of the apparent cooperativity of the actin filament; i.e., it is a measure of the average number of actin sites switched from the closed to the open conformation by the binding of a single S1 head. Thus n is 5−6 for actin·Tm* and 10−12 for actin·Tm*·Tn.

The effect of saturating concentrations of Tn and TnT_2IC on the observed rate constant of S1 binding to actin filaments (k_{LS}) compared to the observed rate constant of the thin filament state change (k_{Fl}) monitored by pyrene excimer fluorescence is shown in Figure 2. The light scattering changes were well described by a single exponential for thin filaments containing Tm, Tm·Tn, or TnT_2IC, and k_{LS} was little affected by the Tn components. The fluorescence changes showed the presence of two components. The slower phase, comprising 30−50% of the total amplitude, had a k_{obs} which was similar to k_{LS} and was ascribed to a fluorescence change due to an S1 which bound close to the pyrene label or to S1 induction of some Tm* binding to actin (Geeves & Lehrer, 1994). The rate of the major fluorescence change was similar for actin·Tm* and actin·Tm*·T_2IC but

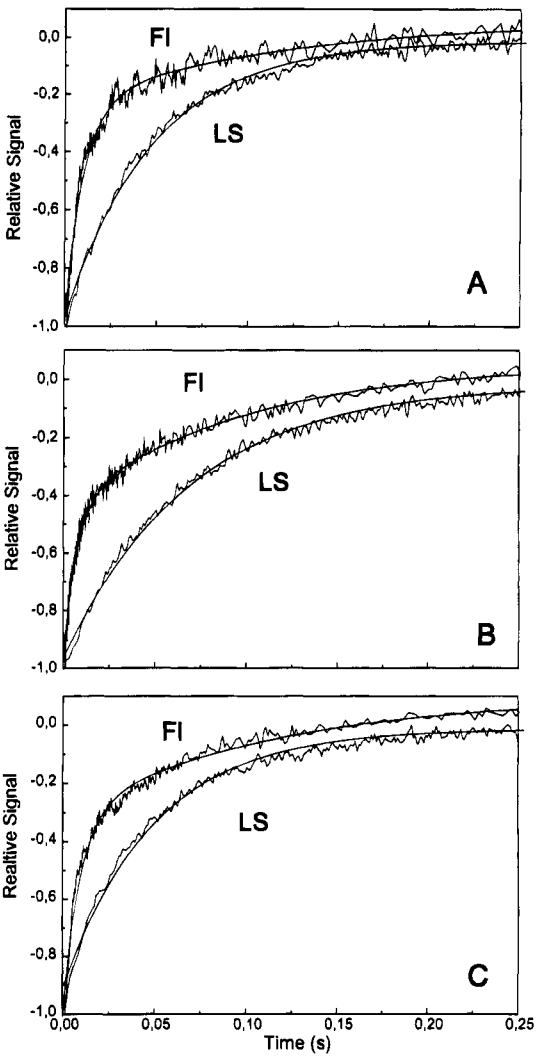

FIGURE 2: Comparison of the effect of TnT_2IC with Tn in the presence of Ca^{2+} on the kinetics of binding (LS) and off-to-on state change (Fl) of the actin·Tm* filament. (A) Tm*; (B) Tm*·Tn; (C) Tm*·TnT_2IC. The best single- (LS) or double-(Fl) exponential fit is shown superimposed for the scaled transients with (A) k_{LS} = 20.4 s^{-1}, k_{1Fl} = 99.1 s^{-1}, k_{2Fl} = 8.85 s^{-1}, fractional ampl = 0.71, n = 4.9; (B) k_{LS} = 14.6 s^{-1}, k_{1Fl} = 140 s^{-1}, k_{2Fl} = 10.5 s^{-1}, fractional ampl = 0.52, n = 9.6; (C) k_{LS} = 20.3 s^{-1}, k_{1Fl} = 107.1 s^{-1}, k_{2Fl} = 9.0 s^{-1}, n = 5.3, fractional ampl = 0.62. Concentrations: 10 μM S1, 1 μM actin, 0.15 μM Tm*, 0.15 μM Tn, 0.25 μM TnT_2IC, 100 μM $CaCl_2$.

was faster when whole Tn was present. The values of the ratio k_{Fl}/k_{LS} show that in contrast to whole Tn, which increases the ratio from 5−6 to 10−12, TnT_2IC has little effect on the ratio (n = 5.3, Figure 2C) and therefore does not significantly affect the size of the cooperative unit.

The reverse experiment of ATP-induced dissociation kinetics of S1 from S1·actin·Tm* in the absence and presence of Tn also provides information on the size of the cooperative unit. Our previous work has shown that light scattering change on adding ATP to S1-loaded filaments was exponential and k_{LS} was linearly dependent on ATP concentration (Geeves & Lehrer, 1994). In contrast, the fluorescence signal did not initially change until the LS signal was almost 80% complete, after which the Fl signal changed rapidly. The last 50% of the fluorescence was approximately exponential, and its rate constant was linearly dependent on ATP concentration. Thus both signals were dependent on ATP concentration and are therefore not rate limited by any

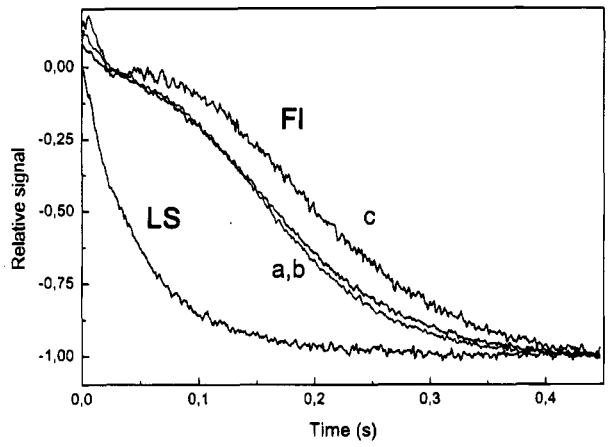

FIGURE 3: Comparison of the effect of TnT_2IC with Tn on the ATP-induced S1 dissociation (LS) and on−off state change kinetics of the S1-saturated thin filament (Fl). (a) Tm*; (b) TnT_2IC·Tm*; (c) Tn·Tm*. The kinetic traces were scaled to the same amplitude and superimposed. Concentrations: 10 mM ATP, 1 μM S1, 1 μM actin, 0.15 μM Tm*, 0.25 μM TnT_2IC, 0.2 μM Tn.

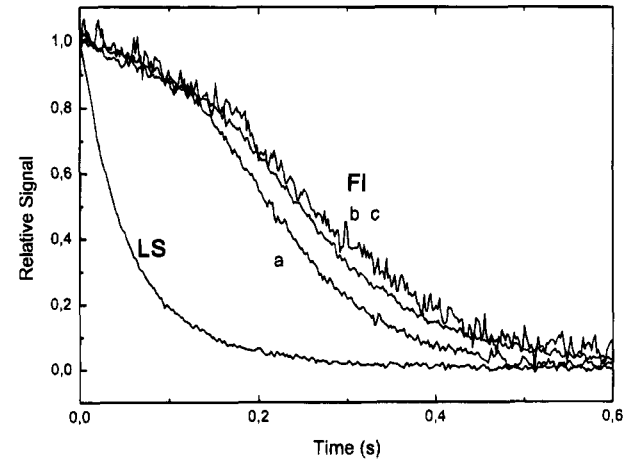

FIGURE 4: Comparison of the effect of TnT_1 with Tn on the ATP-induced S1 dissociation (LS) and on−off state change kinetics of the S1-saturated thin filament (Fl). LS, actin (no regulatory proteins). (a) Tm*; (b) TnT_1·Tm*; (c) Tn·Tm*. The kinetic traces were scaled to the same amplitude and superimposed. Concentrations: 10 mM ATP, 1 μM S1, 1 μM actin, 0.15 μM Tm*, 0.3 μM TnT_1, 0.2 μM Tn.

event subsequent to ATP binding. For this system the size of the lag phase of the Fl compared to the LS is a measure of the size of the cooperative unit, n, i.e., the number of S1s that need to dissociate before a cooperative unit will revert to the closed state.

The light scattering and Tm* fluorescence changes observed on dissociation of S1 from 1 μM actin·Tm*·S1 filaments by addition of 10 μM ATP are shown in Figure 3. The light scattering changes for actin·Tm*, actin·Tm*·Tn, and actin·Tm*·TnT_2IC were all very similar and were well described by a single exponential. In contrast, the fluorescence changes showed a significant lag phase before changing rapidly. As noted earlier the lag in the fluorescence signal was greater for actin·Tm*·Tn compared to actin·Tm*, and the presence of TnT_2IC at the same concentrations used in the experiments shown in Figure 3 did not change the size of the lag in the fluorescence signal. This confirms that TnT_2IC does not appreciably affect the size of the cooperative unit in the actin/Tm thin filament in contrast to the whole Tn complex.

Effects of TnT_1 on the Kinetics of Binding S1 to Actin·Tm. The previous results suggested that the TnT_2IC part of Tn plays no role in increasing the size of the actin·Tm cooperative unit. To determine if TnT_1 increases n, the ATP-induced dissociation experiment was repeated by replacing TnT_2IC with TnT_1 (Figure 4). The exponential change in the light scattering signal was essentially independent of the proteins associated with actin. The fluorescence signal, however, showed a large lag for actin·Tm* (Figure 4, curve a) which increased when Tn was also present (Figure 4, curve c) as observed previously. When TnT_1 was present (Figure 4, curve b), the lag was intermediate between those of actin·Tm* and actin·Tm*·Tn, demonstrating that, unlike TnT_2IC, TnT_1 does increase the cooperativity of the filament. Increasing the concentration of TnT_1 has no further affect on the size of the lag in the fluorescence signal, suggesting that TnT_1 binds tightly to the filament with a stoichiometry of approximately one TnT_1 per seven actins.

The kinetics of S1 binding to actin·Tm*·TnT_1 showed that the scattering signal was a single exponential and k_{LS} was independent of the presence of Tn or TnT_1 (Figure 5). The pyrene fluorescence signal was again composed of two components. The observed rate constant of the principal faster component increased when TnT_1 was present and increased further in the presence of whole Tn. The ratio, k_{Fl}/k_{LS}, was 5−6 for actin·Tm* (Figure 5A), increased to 8−9 for actin·Tm*·TnT_1 (Figure 5B), and was 10−12 for actin·Tm·Tn (Figure 5C).

Our previous studies have shown that the addition of Tn to the Tm·actin filament has two effects. It introduces Ca^{2+} dependence to the thin filament equilibria and increases the size of the cooperative unit (Geeves & Lehrer, 1994). In this work troponin was split into two parts: a part that interacts with the head−tail region of neighboring Tm molecules on actin and a part that interacts with Tm about 130 Å away from the head-tail region. Each part was shown to have one of the two properties that intact troponin imparts to the thin filament.

In a recent publication, troponin was reconstituted by replacing TnI with a TnI-mutant which does not bind to TnT and shown to have a Ca^{2+}-sensitive ATPase activity similar to that of wild type troponin (Potter et al., 1995). Although the authors proposed a Ca^{2+}-dependent TnC−TnT interaction, an alternate explanation is possible based on our work which shows that troponin contains two separate regions that function relatively independently. Thus, in the absence of Ca^{2+}, mutant TnI−TnC, which is unable to interact with bound TnT, can maintain Tm·actin mainly in the "off state". In the presence of Ca^{2+}, where the TnI−TnC interaction with actin·Tm is weakened, TnT is responsible for the activation due to the increased n produced in its presence.

CONCLUSIONS

(1) By proteolytic cleavage of TnT in the intact Tn complex, we have separated Tn into two fragments, TnT_2IC and TnT_1, and investigated effects of each component on the kinetics of the S1−actin·Tm interaction and resultant changes in thin filament state. Both fragments bind tightly to the filament with a stoichiometry of close to one per seven actins.

(2) In the absence of Ca^{2+}, TnT_2IC inhibits the initial binding of S1 to a similar extent as whole Tn while the

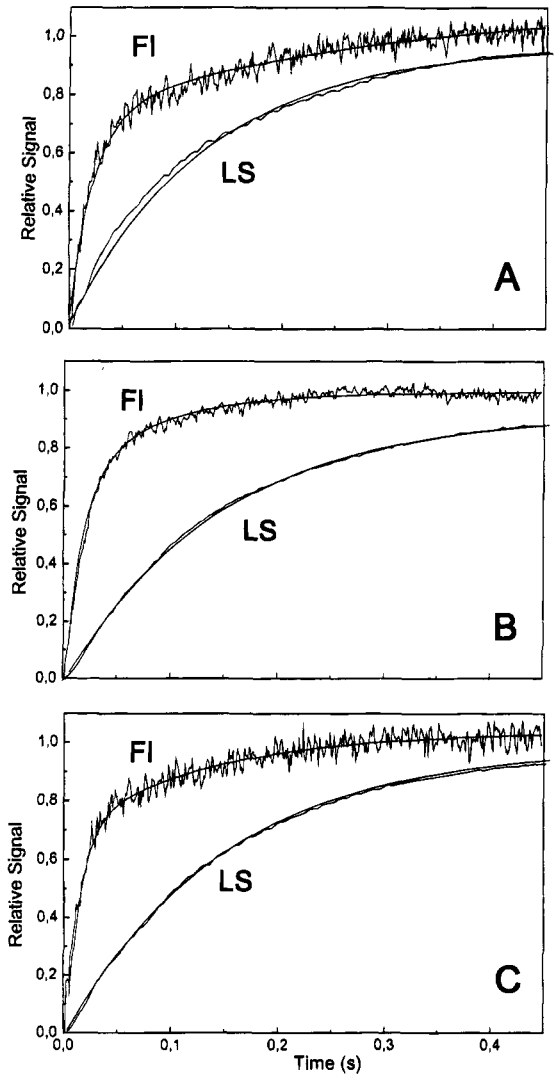

FIGURE 5: Comparison of the effects of TnT_1 with Tn on the kinetics of S1 binding (LS) and the off–on state change of the actin·Tm* thin filament. (A) actin·Tm*; (B) actin·Tm*·TnT_1; (C) actin·Tm*·Tn. The best single- (LS) or double-(Fl) exponential fit is shown superimposed for the scaled transients with (A) k_{LS} = 7.3 s^{-1}, k_{1Fl} = 45.5 s^{-1}, k_{2Fl} = 5.6 s^{-1}, fractional ampl = 0.64, n = 6.2; (B) k_{LS} = 6.9 s^{-1}, k_{1Fl} = 63.2 s^{-1}, k_{2Fl} = 12.6 s^{-1}, fractional ampl = 0.69, n = 9.1; (C) k_{LS} = 6.8 s^{-1}, k_{1Fl} = 79.6 s^{-1}, k_{2Fl} = 7.62 s^{-1}, fractional ampl = 0.71, n = 11.7. Concentrations: 5 μM S1, 1 μM actin, 0.15 μM Tm*, 0.3 μM TnT_1, 0.2 μM Tn.

apparent size of the cooperative unit remains the same as in the absence of Tn. The small differences between the binding reactions observed for Tn compared to TnT_2IC in the absence of Ca^{2+} (shown in Figure 1) may be accounted for by a somewhat smaller apparent cooperative unit size for TnT_2IC compared to whole Tn. Thus TnT_2IC behaves like Tn by sterically blocking initial S1 binding, either itself or via Tm (Lehrer, 1994). TnT_2IC may therefore be used to study effects of Ca^{2+} on regulation without the additional complexity of increased cooperativity.

(3) TnT_1 binds to actin·Tm to increase the cooperative unit size without affecting the rate of S_1 binding or introducing a blocked state.

(4) Since intact Tn exhibits both the properties of TnT_2IC and TnT_1, we have succeeded in showing that the Ca^{2+}-dependent inhibition of S1 binding in the weak state is a property of the TnT_2IC region of Tn and the Ca^{2+}-independent increase in the size of the cooperative unit is a property of the TnT_1 region of TnT. This suggests that the two parts of TnT act independently.

ACKNOWLEDGMENT

We appreciate the excellent technical assistance of Ms. Nancy Adamek and Mr. Xiaofeng Zhou.

REFERENCES

Brisson, J. R., Golosinska, K., Smillie, L. B., & Sykes, B. D. (1986) *Biochemistry 25*, 4548–4555.
Flicker, P., Phillips, G. N. J., & Cohen, C. (1982) *J. Mol. Biol. 162*, 495–501.
Geeves, M. A., & Halsall, D. J. (1987) *Biophys. J. 52*, 215–220.
Geeves, M. A., & Lehrer, S. S. (1994) *Biophys. J. 67*, 273–282.
Greene, L., & Eisenberg, E. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 2616–1620.
Head, J. G., Ritchie, M. D., & Geeves, M. A. (1995) *Eur. J. Biochem. 227*, 694–699.
Heeley, D. H., Golosinska, K., & Smillie, L. B. (1987) *J. Biol. Chem. 262*, 9971–9978.
Ishii, Y., & Lehrer, S. S. (1990) *Biochemistry 29*, 1160.
Leavis, P. C., & Gergely, J. (1984) *CRC Crit. Rev. Biochem. 16*, 235–305.
Lehrer, S. S. (1994) *J. Muscle Res. Cell Motil. 15*, 232–236.
Lehrer, S. S., & Kerwar, G. (1972) *Biochemistry 11*, 1211–1217.
Lehrer, S. S., & Morris, E. P. (1982) *J. Biol. Chem. 257*, 8073–8080.
McKillop, D. F. A., & Geeves, M. A. (1993) *Biophys. J. 65*, 693–701.
Morris, E. P., & Lehrer, S. S. (1984) *Biochemistry 23*, 2214–2220.
Ohtsuki, I. (1979) *J. Biochem. (Tokyo) 86*, 491–497.
Pearlstone, J. R., & Smillie, L. B. (1982) *J. Biol. Chem. 257*, 10587–10592.
Potter, J. D., Sheng, Z., Pan, B.-S., & Zhao, J. (1995) *J. Biol. Chem. 270*, 2557–2562.
Spudich, J., & Watt, S. (1971) *J. Biol. Chem. 246*, 4866–4871.
Trybus, K. M., & Taylor, E. W. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 7209–7213.
Weeds, A. G., & Pope, B. (1977) *J. Mol. Biol. 111*, 129–157.
Williams, D. L., Greene, L. E., & Eisenberg, E. (1988) *Biochemistry 27*, 6987–6993.

BI9520545