Biochemistry

 Volume 36, Number 44 November 4, 1997

Articles

Actin-Tropomyosin Activation of Myosin Subfragment 1 ATPase and Thin Filament Cooperativity. The Role of Tropomyosin Flexibility and End-to-End Interactions†

Sherwin S. Lehrer,*,‡ Nina L. Golitsina,‡ and Michael A. Geeves§

Muscle Research Laboratories, Boston Biomedical Research Institute, Boston, Massachusetts 02114, and Max Planck Institut fur Molekulare Physiologie, D-44139, Dortmund, Germany

Received June 30, 1997; Revised Manuscript Received August 19, 1997⊗

ABSTRACT: Tropomyosin (Tm) bound to actin induces cooperative activation of actomyosin subfragment 1 (actin-S1) ATPase, observed as a sigmoid ATPase vs [S1] dependence. The activation is much steeper for gizzard muscle Tm (GTm) than for rabbit skeletal Tm (RSTm). To investigate if this greater cooperativity is due to increased communication between GTms along the thin filament, we studied effects of S1 binding on the state of actin-Tm using the fluorescence of pyrene-labeled Tm. Kinetic and equilibrium studies provided values for *n*, the apparent cooperative unit size [Geeves, M. A., and Lehrer, S. S. (1994) *Biophys. J. 67*, 273]. We report comparative studies of Tm-actin-S1 ATPase with values of *n* using GTm, RSTm, and 5aTm, a $^1/_7$ shorter nonmuscle Tm from rat fibroblast cells [Pittenger, M. F., et al. (1994) *Curr. Opin. Cell Biol., 6*, 96]. 5aTm and GTm produce similar cooperative activation of actin-S1 ATPase and have similar *n* values that are 2-fold greater than RSTm, indicating a correlation between ATPase activation and *n* value. This appears to be due to the similarity of the C-terminal amino acid sequences of 5a and GTm which produce strong end-to-end interactions. The results are discussed in terms of a continuous flexible Tm strand on the actin filament.

The presence of tropomyosin (Tm)[1] on actin thin filaments confers cooperativity to the interaction between myosin subfragment 1 (S1) and actin. This can be observed in the S1 dependence of the actin activation of S1 ATPase (*1*) and in the binding of S1 to actin (*2*). The nature and extent of thin filament cooperativity has been subject of numerous in vitro studies and interpretations (*1*, *3*−*7*). These studies indicate that the Tm-containing thin filament exists in at least two states which differ in the strength and form of its interaction with S1−nucleotide complexes. Recent structural studies suggest that Tm, which interacts with seven actin subunits, can occupy two different positions on the actin surface, one of which partially obstructs the S1 binding site on actin (*8*, *9*), providing a possible structural model for the two states. The presence of Tn in the absence of Ca^{2+} introduces a third state (*10*), which obstructs a larger fraction of the S1 binding site. Here, we will consider the simpler actin-Tm filament since the presence of Tm is sufficient to observe these cooperative effects. Our goal is to understand the role of the transition between the two functional states of actin-Tm in regulating the actin-myosin interaction. Tm

† Supported by NIH Grants AR 41637 and HL 22461 and NATO.
* Author to whom correspondence should be addressed. Tel: 617-742-2010. Fax: 617-523-6649. Email: Lehrer@bbri.harvard.edu.
‡ Boston Biomedical Research Institute.
§ Max Planck Institut fur Molekulare Physiologie. Tel: 49-231-1206-367. Fax: 49-231-1206-228. Email: Geeves@mpi-dortmund.mpg.de.
⊗ Abstract published in *Advance ACS Abstracts*, October 1, 1997.
[1] Abbreviations: RSTm and GTm, rabbit skeletal and gizzard tropomyosin; Tm*, pyrene-labeled Tm; S1, myosin subfragment 1; *n*, cooperative unit size.

S0006-2960(97)01568-7 CCC: $14.00

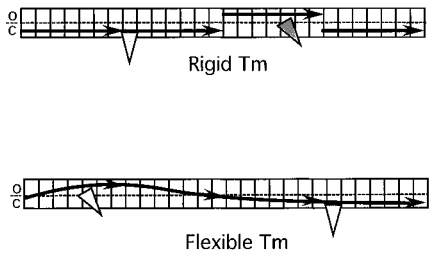

FIGURE 1: Schematic diagram of Tm fluctuating between the closed (off) and open (on) thin filament states. Rigid Tm molecule with $n = 7$. An end-to-end interaction parameter, Y, allows for communication between Tms (*4*). Semiflexible Tm illustrated for strong end-to-end interaction. Here, n can have variable values from below 7 to the number of actin subunits in a filament.

forms a continuous structure along actin and strong end-to-end interactions may provide a significant amount of the binding energy of Tm to actin (*6*, *11*). For a continuous Tm strand, it is not clear what model to use for the size of the functional unit that changes its state on the actin surface. If each Tm is treated as a rigid rod, the cooperative unit for the transition will be the actin$_7$-Tm unit (Figure 1) and each unit will have a defined probability for each of the two states. Additional cooperativity can be modeled using a variable Tm end-to-end interaction parameter (*4*). If this interaction is very strong, the whole rigid strand could, in principle, change state in a concerted transition. This rigid Tm model presents certain conceptual problems when the Tm end-to-end interaction provides a substantial fraction of the binding energy because two strong interactions would be broken when one Tm changes state. Since the free energy change of the transition is small (*12*), Tm−Tm interactions do not appear to be broken. Alternatively, in the presence of strong end-to-end interactions, one can consider Tm as a flexible dynamic filament (*13*−*17*) in equilibrium between open and closed positions (Figure 1). The persistence length of the Tm and the amplitude of the deviation from its mean position determine the extent to which changes in the position of Tm over a single actin are communicated to adjacent actins. On binding an S1 to an actin subunit in the open state, several actin subunits on each side of the S1-bound actin would be trapped in the open state.

In our recent work, we have defined n, the apparent cooperative unit size of the thin filament, as the average number of actin subunits trapped in the open state by the binding of one S1. This definition is independent of either of the above models for the actin-Tm filament. Any parameter which changes the interaction between adjacent Tms could also affect the communication. Thus, for example, the binding of TnT$_1$, the N-terminal chymotryptic fragment of troponin T, to the Tm−Tm overlap region increases n (*18*). The length of Tm, the relative strength of end-to-end interactions for different Tms and changes in flexibility by binding other proteins could also change n.

Use of Tms expressed in different tissues and from different species provides a way to test the influence of length, flexibility, and end-to-end interactions on the properties of reconstituted thin filaments. The principal difference between the α and β isoforms of rabbit muscle skeletal Tm (RSTm) and the corresponding gizzard smooth muscle Tms (GTm) is in the sequence of the C-terminal nine amino acids (Table 1). This results in GTm having much stronger end-to-end interactions than RSTm as measured by specific viscosity at low salt (*19*, *20*). GTm induces greater cooperative effects in actin-myosin subfragment 1 (actin-S1) ATPase than rabbit skeletal Tm (RSTm) (*21*). This greater cooperativity caused by GTm could result from increased communication between Tms along the thin filament. Fibroblast 5a Tm is shorter by approximately one actin binding site than GTm and RSTm, has an almost identical C-terminus to GTm, and a similar N-terminal sequence to both GTm and RSTm with an N-terminal extension of five amino acids (Table 1) (*22*).

Table 1: Comparison of 10 Amino Acid Residues from the N- and C-Termini of the Isoforms of Rabbit Skeletal Tm (RS), Gizzard Muscle Tm (G), and Rat Recombinant Fibroblast 5aTm (5a)[a]

Tm	N-terminus	C-terminus
Tm	N-TERMINUS	C-TERMINUS
αRS	MDAIKKKMQM	DHALNDMTLI
βRS	----------	-N----I--L
αG	----------	-QT-LELNNM
βG	-E--------	-QT-LELNNL
5a	agsssLE-VRR-IRS	-QT-LELNNM

[a] The dashes indicate identical residues compared to the sequence of aRSTm.

We now report comparative studies of Tm-actin-S1 ATPase and S1 binding effects on Tm-actin state using GTm, RSTm, and 5aTm. 5aTm and GTm produce similar cooperative activation of actin-S1 ATPase and have similar n values, in both cases greater than RSTm, despite the smaller length of 5aTm. We suggest that this is due to the similarity of the C-terminal amino acid sequences of 5a and GTm which produce strong end-to-end interactions, allowing for the flexibility of Tm to be manifested.

EXPERIMENTAL PROCEDURES

Rabbit skeletal actin was prepared from an acetone powder of rabbit muscle and purified by cycles of polymerization/depolymerization (*23*, *24*). Chicken gizzard Tm (GTm) and rabbit skeletal Tm (RSTm) were purified by isoelectric precipitation and ammonium sulfate fractionation (*1*, *21*). For the fluorescence experiments, Tm was labeled with pyrene iodoacetamide as described earlier (*25*). RSTm which consists predominantly of a 60/40 mixture of αα/αβ isoforms (*26*), was used without further fractionation because preliminary experiments showed that the properties of αα and αβ RSTm were very similar. Native GTm which consists of 100% αβ (*27*) was used for the ATPase and viscosity studies. The smooth muscle α and β amino acid sequences are different from the striated due to alternative splicing (*28*). For the fluorescence studies, pyrene-ααGTm was used by separating the labeled α-chain from the labeled β-chain with reversed phase HPLC using a semimicro biphenyl column (Vydac) with a 40 to 60% acetonitrile/TFA gradient (*29*). Fibroblast recombinant 5aTm, in the form of a partially purified pH 4.6 pellet, was obtained from Drs. M. Gimona and D. Helfman, Cold Spring Harbor Labs. The 5aTm was purified by HPLC as for the pyrene-GTm before labeling.

After lyophilization, the pooled HPLC fractions were dissolved in 5 M GuHCl and 10 mM Hepes buffer, pH 7.0, and renatured by dialyzing against 0.5 M NaCl and 20 mM Hepes buffer. The concentration of the labeled Tms was determined by BCA-protein assay (Pierce) using unlabeled Tm as a standard. The concentration of pyrene bound to Tm was determined with $e_{344nm} = 2.2 \times 10^4$ M^{-1}. The labeling ratio, pyrene/Tm, was between 0.5 and 0.6 for GTm, 5aTm, and RSTm. S1 and HMM were prepared by chymotryptic digestion of myosin (*30*).

ATPase measurements were performed as described by White (*31*) with some modifications. Actin, Tm, and ATP were incubated in 30 mM NaCl, 5 mM $MgCl_2$, and 10 mM Hepes, pH 7.5, in 250 μL tubes at 25 °C and initiated with S1. The reactions were quenched at suitable times with 3.3% SDS and developed with ammonium molybdate/ferrous sulfate solution prepared fresh in dilute sulfuric acid, as described, and read at 650 nm within $^1/_2$ h. The rates were shown to be linear over the times used for this study. Duplicate measurements gave values within 10%.

Stopped flow kinetic measurements were performed as outlined previously (*12*).

Viscosity measurements were performed on 0.5 mL Tm solutions in a Cannon-Manning viscometer with a buffer flow time of 60 s in a water bath at 25 °C. Tm solutions at 0.5 mg/ml were dialyzed vs 2 mM Hepes buffer before measurements of flow times which were the averages of at least three measurements at each [salt]. The salt concentration was increased sequentially by adding 5 M NaCl to the viscometer.

Steady-state fluorescence data were obtained with a SPEX Fluorolog 2/2/2 photon-counting fluorometer (Edison, NJ) in the ratio mode with a 2.5 nm band-pass for both excitation and emission on samples incubated in the 25° thermostated housing. Titrations were carried out with excitation at 340 nm and emission at 343 nm to monitor light scattering. A 450 nm cut-on KV Schott filter placed in front of a second photomultiplier at 90 °C to the excitation was located close to the sample to simultaneously measure fluorescence. Before titrations, the samples were clarified by centrifugation at low speed for 10 min.

RESULTS

ATPase Studies. The effect of RSTm on the S1 concentration dependence of actin activated S1 ATPase has previously been studied (*1*). A typical ATPase data set for S1, actin-S1, and actin-Tm-S1 with the three different Tms is shown in Figure 2. In the absence of actin, the k_{cat} of the S1 ATPase is low (0.05 s^{-1}) and the observed rate of Pi production is linearly dependent upon the S1 concentration. At a fixed, moderate actin concentration (shown here for 4 uM actin), the actin-S1 ATPase also increases linearly with S1 concentration, indicating that the activation by actin is also independent of [S1] at these low concentrations where very little of the S1 is bound to actin. (At higher concentrations a hyperbolic dependence of ATPase on [S1] occurs approaching V_{max} at actin saturation.) In contrast, when RSTm is bound to actin, a sigmoidal increase in S1 dependence of ATPase is produced which results in inhibition and activation of acto-S1 ATPase at very low and high [S1], i.e., a variable activation over the S1 alone value. This indicates that RSTm produces cooperative effects in the actin

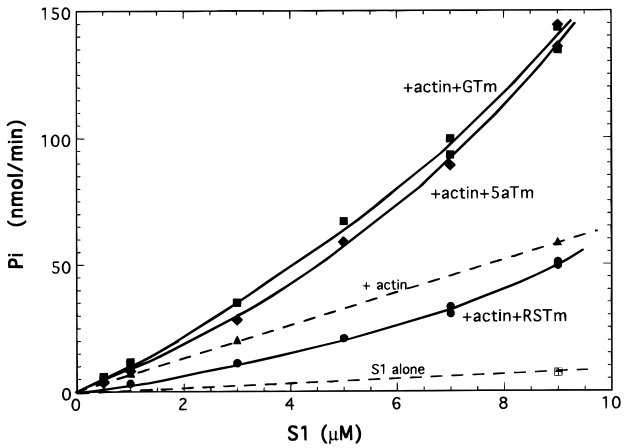

FIGURE 2: ActoS1 ATPase vs [S1]. Cooperative effects of S1 in the presence of RSTm, GTm, and 5aTm. Conditions: [actin] = 4 μM, [Tm] = 1.2 μM in 30 mM NaCl, 5 mM $MgCl_2$, 2 mM ATP, 10 mM Hepes, pH 7.5.

activation of the S1 ATPase. Although the inhibition at low [S1] reaches similar values for GTm if the [Mg^{2+}] is high enough (*21*, *32*), the change to activation of the ATPase by actin-GTm takes place at lower [S1] indicating greater cooperativity (*21*) (Figure 2). The data in Figure 2 are not precise enough at low [S1] to show the inhibition of the ATPase but clearly demonstrate the nonlinear activation of the S1 ATPase. Rat fibroblast 5aTm, which is $^6/_7$ as long as GTm and RSTm, activates acto-S1 ATPase in a similar manner to GTm (Figure 2) suggesting that there is no simple correlation between the degree of cooperativity and Tm length, i.e., between the size of the structural unit and cooperativity.

Fluorescence Studies. Previous studies have shown that the excimer fluorescence of pyrene-labeled Tm (Tm*) can report the conformational change associated with the closed/open equilibrium of the actin-Tm filament induced by S1 binding (*12*, *25*). Values for *n*, the apparent cooperative unit size, which is the probability that the random binding of one or more S1s can trap *n* actin subunits of actin-Tm in the open state, can be obtained from equilibrium and kinetic measurements.

In an equilibrium titration of S1 into actin-Tm*, the light scattering (LS) changes show that S1 binds stoichiometrically (shown by the break in the LS curve, Figure 3A). The fluorescence (Fl) changes are complete before the actin is saturated with S1, indicating that the binding of S1 causes the Tm to change state before all of the actin sites are occupied by S1. The value of *n* was obtained by plotting the fractional fluorescence change vs the fractional binding and comparing the experimental curve with model curves of different *n* (Figure 3B). The curves are generated from the simple statistical equation, $f_{open} = 1 - (1 - f_{bound})^n$, which assumes that the random binding of one or more S1s to *n* actin subunits, produces the total fluorescence change due to Tm switching from the closed (off) to the open (on) state. Although the same tight binding of S1 to actin was seen for actin-GTm* and actin-RSTm* as for actin-5aTm*, and the total fluorescence changes were about the same, the shape of the fluorescence profiles were different. The curves were best described for $n = 5$ for RSTm and $n = 8-10$ for both GTm* and 5aTm* (Figure 3B). The slight deviation of the data from the model at lower saturation is apparently due to the presence of some ATP which reduced the initial binding

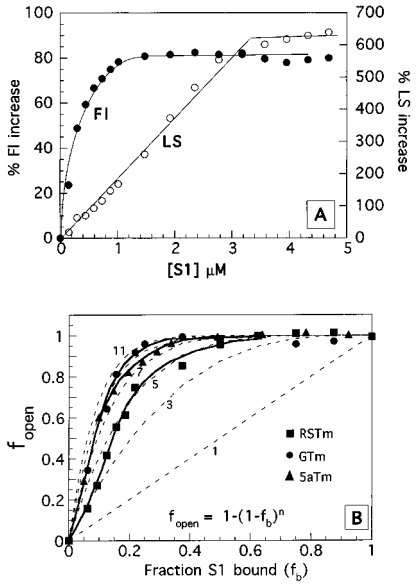

FIGURE 3: (A) Titration of 5aTm*−actin with S1. The pyrene excimer fluorescence (Fl) monitors the fraction in the open (on) state associated with the fraction S1 bound monitored by light scattering (LS). (B) Comparison of equilibrium S1 titrations of RSTm*, GTm*, and 5aTm* bound to actin with statistical model for indicated *n*. The fraction bound, f_b, and fraction open, f_{open}, were obtained from Fl and LS changes, respectively (see panel A). Conditions: [actin] = 3.5 μM, [5aTm*] = 0.5 μM in 50 mM NaCl, 5 mM $MgCl_2$, 20 mM Hepes, pH 7.4.

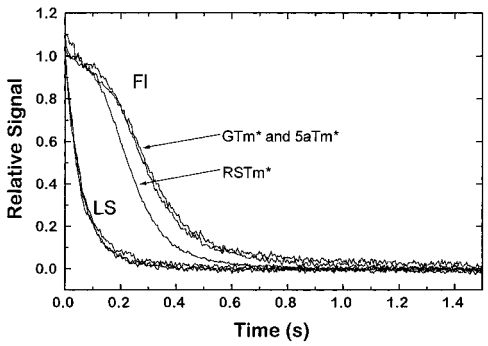

FIGURE 4: Kinetics of ATP-induced dissociation of S1 from actin−RSTm, actin−GTm, and actin−5aTm. Conditions same as for Table 1 except [S1] = 1 μM and [ATP] = 10 μM.

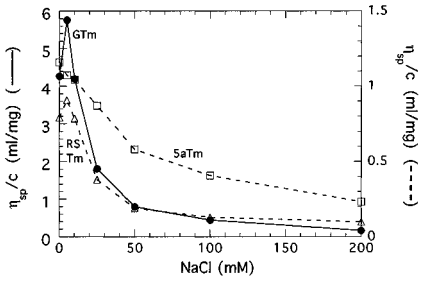

FIGURE 5: Salt dependence of specific viscosity for GTm (●), 5aTm (□) and RSTm (△). Conditions: [Tm] = 0.5 mg/mL, in 2 mM Mops buffer pH 7.0, 24 °C.

but which was hydrolyzed on addition of S1. The agreement of the data with the shape of the theoretical curve indicates that S1 does bind randomly to actin−Tm since nonrandom binding would produce different shaped fluorescence curves.

The kinetics of binding of S1 to actin-Tm* and accompanying thin filament state change were also monitored with light scattering (LS) and fluorescence (Fl), respectively. One would expect that the Fl change would temporally lead the LS change if the binding of 1 S1 affects several actin subunits. Previous work has indeed shown that the ratio of the respective observed exponential rate constants, k_{Fl}/k_{LS} is equal to *n* (*12*). From these ratios, we obtained $n = 7-10$ for RSTm and 15−20 for both GTm and 5aTm (Table 1). Although the reason for the greater value of *n* than previously observed for actin-RSTm* (*12*) is not clear, the values for both GTm and 5aTm were about 2× greater, as observed in the equilibrium titrations.

The complementary kinetic experiment of the ATP-induced dissociation of S1 from S1-actin-Tm* also provides information on the relative size of *n* (*12*). Random binding of ATP to actin bound S1 causes rapid dissociation, giving rise to a scattering change. However, a few remaining S1s that have not yet dissociated can keep the actin-Tm* in the open (on) state, and a lag is expected in the Fl decay that persists after most of the LS has exponentially decayed, the size of which depends on *n*. The experiments show that the fluorescence lag is about the same for GTm and 5aTm and greater than RSTm (Figure 4) in agreement with the greater *n* obtained in the above experiments.

Viscosity Studies. Early studies have indicated that specific viscosity is a measure of Tm end-to-end polymerization at low salt (*33*) and many studies have been made over the years that show that troponin increases the viscosity greatly, in agreement with its overlapping the head-tail Tm junction. However, viscosity measurements should be interpreted with caution since it is possible that there are side-by-side Tm interactions at high protein concentrations similar to those seen in Tm paracrystals, which may affect viscosity. With this in mind, viscosity measurements were made on similar concentrations of the three Tms at increasing salt concentrations (Figure 5). It can be seen that, at low salt, GTm had the greatest viscosity and 5aTm, rather than behaving similar to GTm, had about the same viscosity as RSTm. It is interesting to note, however, that the viscosity of 5aTm had a different salt dependence; RSTm and GTm both required 0.02 M salt for depolymerization, but 0.1 M salt was required to half depolymerize 5aTm. We, as yet, have no explanation for the salt dependence of 5aTm viscosity.

DISCUSSION

We have previously shown that, for Tm-containing actin filaments, it is possible to obtain values for the apparent cooperative unit size, *n*, the average number of actin subunits activated by the binding of a single S1, using a conformationally sensitive fluorescence probe on Tm. The present study provides evidence for a relationship between the effects that Tm produces on the sigmoidal S1 concentration dependence of actin-S1 ATPase and *n*. We found that the value of *n* is 2-fold greater for GTm and 5aTm compared to RSTm and this correlates with the ability of GTm- and 5aTm-actin to more readily activate the S1 ATPase than RSTm-actin at the same S1 concentration.

It should be pointed out that the greater activation by Tm at a given [S1] does not imply that Tm changes the acto−S1 V_{max}, but rather that due to the greater cooperativity, the V_{max} is reached at lower [S1]. In fact, Williams et al. showed that V_{max} for actin-Tm-S1 ATPase was about the same as in the absence of Tm for both rabbit skeletal Tm and gizzard smooth Tm using NEM S1 as an activating ligand (*32*, *42*).

These results are consistent with a single tightly bound S1 switching on a larger number of actin subunits because

Table 2: Observed LS and Fl Rate Constants on Binding S1 to Actin−Tm*[a]

Tm*	k_{LS} (s^{-1})	k_{Fl} (s^{-1})	$n = (k_{Fl}/k_{LS})$
RSTm	11.6	92.2	7−10
GTm	12.8	261	15−20
5aTm	12.9	268	15−20

[a] Conditions: [S1] = 5 mM, [actin] = 1 mM, [Tm] = 0.2 mM, 140 mM KCl, 5 mM $MgCl_2$, 20 mM Mops buffer, pH 7.0, 20 °C.

of greater communication between Tms along the filament. We suggest that there are sufficient R-type S1-actin complexes present during steady state ATPase to activate the system. The observation that GTm and the $^1/_7$ shorter 5aTm are indistinguishable while RSTm is less cooperative suggests that cooperativity is not simply related to the number of actin subunits with which a single Tm interacts. Our central question is therefore how to interpret the cooperativity of the closed-open (on-off) transition characterized by the apparent cooperative unit size *n*, in terms of properties of Tm.

Cooperativity between Adjacent Structural Units. Previous studies have shown that troponin increases the cooperative activation of the S1 ATPase by actin-RSTm (*1*) and increases the end-to-end polymerization of RSTm (*34*). Fluorescence studies with pyrene-Tm, similar to those presented here, have indicated that troponin, and in particular the troponin T1 fragment of troponin T, increases *n* about 2 fold (*18*). TnT1 overlaps adjacent Tm molecules along the actin filament (*35*, *36*), and therefore, these effects of troponin are consistent with a model in which an increase in the interaction between Tms along the filament results in an increase in both the apparent cooperative unit size and the degree of cooperativity in actin-S1 ATPase. Thus, a similar correlation between the cooperativity in the actin-S1 ATPase and *n* for GTm and 5aTm could be explained by a stronger interaction between these Tms.

The end-to-end interactions between Tms are dependent on the amino acid sequence in the overlap region which consists of about 10 amino acid residues from the N- and C-termini (*14*). The C-terminal 10 amino acid residues of the αGTm and 5aTm isoforms are identical, and *β*GTm only differs by one conservative change at the terminus, Leu for Met (Table 2). In contrast, the C-termini of αRSTm and *β*RSTm, which are similar to each other, are quite different from the smooth and nonmuscle Tm isoforms. The N-terminal 10 amino acids of both isoforms of the smooth and striated Tms are almost identical with only one conservative change for *β*GTm, Glu for Asp. Thus, the native α*β* GTm heterodimer should have similar end-to-end interaction properties as 5aTm, but quite different from the αα/α*β* native mixture of RSTm. It is interesting to note that the N-terminal sequence of 5aTm contains six additional residues before beginning a sequence of 10 amino acid residues that are quite similar to the others, e.g., it contains the conserved triplet of basic amino acid residues at complementary positions. Thus, the difference in Tm−Tm interactions between GTm and RSTm is due primarily to the differences in the C-terminus. If the six residue extension at the N-terminus of 5aTm plays no major role in the Tm−Tm interaction on actin, 5aTm should have similar Tm−Tm interactions as GTm. Thus, the similarities in cooperativity observed for GTm and 5aTm and the differences with RSTm may be all accounted for by the differences in the RSTm C-terminus.

The observation of a low specific viscosity for 5aTm at low salt despite its smooth-muscle type C-terminus suggests that the six residue N-terminal extension of 5aTm may decrease end-to-end interactions for Tm alone without affecting Tm intercommunication while bound to actin. A fusion Tm that contained an 80 residue extension at the N-terminus had low salt viscosity but bound well to actin (*37*), a process which requires strong end-to-end interactions (*6*, *11*). However, a three or two residue N-terminal extension maintains the properties of the native molecule (*38*). Clearly, further studies are required to determine the relationship between number and type of amino acid extension and it affects on end-to-end interactions.

The original treatment of thin filament cooperativity (*4*) used a fixed cooperative unit size of 7 based on the structural unit size for a RSTm and two variable parameters: an equilibrium constant between the on and off states of the thin filament, and an interaction parameter between adjacent cooperative units. For the three Tms studied here differences in the interaction parameter would be primarily determined by the C-terminal sequence and should be similar for GTm and 5aTm. However, as the structural unit is smaller for 5aTm (by $^1/_7$ or 14%), a smaller value of *n* is expected for 5aTm compared to GTm unless the interaction parameter overcompensates, an improbable situation in view of the similarity of the amino acid sequences at the termini.

The alternative to this rigid Tm model is that the apparent cooperative unit and the cooperativity of the ATPase is not simply determined by the length of the Tm and the strength of the end-to-end interactions, but that the flexibility of Tm must be taken into account.

Role of Flexibility in Tm. A variety of studies of indicated that Tm is a semiflexible molecule both for molecular Tm alone and when bound to actin (*13*−*17*, *19*, *39*). Also, it has been proposed that the binding site(s) of Tm on the actin filament are not highly specific (*40*). If the end-to-end interactions between Tms along an actin filament are sufficiently strong, Tm can be considered a continuous semiflexible strand along the filament.

In view of the above considerations and the small value for the equilibrium constant between the open and closed Tm-actin states, (0.2) (*12*), Tm may be expected to change position on the actin surface with relatively small changes in the energy of interaction and thermal fluctuations could cause significant rapid movements of Tm over the actin surface. RSTm has been shown to have a persistence length of about 1300 Å in solution (*13*). This suggests that Tm could bend enough in 2 molecular lengths (800 Å and $n = 14$) to move 5−10 Å between the closed and open positions, values estimated from structural studies (*9*, *41*).

The data presented here suggest that Tm flexibility is a determinant of the apparent cooperative unit size, *n*, the number of actin subunits/S1 trapped in open state. Since the persistence length of Tm is much greater than the molecular length, when the end-to-end interactions are strong, *n* will be greater than 7, the number of actin subunits interacting with a Tm molecule. When the end-to-end interactions are weak, *n* will be approximately equal to or somewhat less than 7. Molecular genetic approaches may allow the roles of Tm length, end-to end interactions, and flexibility to be examined more precisely.

ACKNOWLEDGMENT

The excellent technical assistance of Ms. Minhua Chai and Ms. Nancy Adamek is greatly appreciated. We also would like to thank Drs. Mario Gimona and David Helfman of Cold Spring Harbor Laboratories, for the generous gift of recombinant rat fibroblast 5aTm and to Dr. Robin Maytum for a sample of purified recombinant 5aTm.

REFERENCES

1. Lehrer, S. S., and Morris, E. P. (1982) *J. Biol. Chem. 257*, 8073−8080.
2. Greene, L., and Eisenberg, E. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 2616−1620.
3. Bremel, R. D., Murray, J. M., and Weber, A. (1972) *Cold Spring Harbor Symp. Quant. Biol. 37*, 267−275.
4. Hill, T., Eisenberg, E., and Greene, L. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 3186−3190.
5. Nagashima, H., and Asakura, S. (1982) *J. Mol. Biol. 155*, 409−428.
6. Hill, L. E., Mohegan, J. P., Butters, C. A., and Tobacman, L. S. (1992) *J. Biol. Chem. 267*, 16106−16113.
7. Pan, B.-S., Gordon, A. M., and Luo, Z. (1989) *J. Biol. Chem. 264*, 8495−8498.
8. Lehman, W., Craig, R., and Vibert, P. (1995) *Biophys. J. 68*, A366.
9. Vibert, P., Craig, R., and Lehman, W. (1997) *J. Mol. Biol. 266*, 8−14.
10. McKillop, D. F. A., and Geeves, M. A. (1993) *Biohys. J. 65*, 693−701.
11. Wegner, A. (1979) *J. Mol. Biol. 131*, 839−853.
12. Geeves, M. A., and Lehrer, S. S. (1994) *Biophys. J. 67*, 273−282.
13. Hvidt, S., Ferry, J. D., Reolke, D. L., and Greaser, M. L. (1983) *Macromolecules 16*, 740−745.
14. Phillips, G. N., Fillers, J. P., and Cohen, C. (1986) *J. Mol. Biol. 192*, 111−131.
15. Swenson, C. A., and Stellwagen, N. C. (1989) *Biopolymers 28*, 955−963.
16. Szczesna, D., and Fajer, P. G. (1995) *Biochemistry 34*, 3614−3620.
17. Phillips, G. N. J., and Chacko, S. (1996) *Biopolymers 38*, 89−95.
18. Schaertl, S., Lehrer, S. S., and Geeves, M. A. (1995) *Biochemistry 34*, 15890−15894.
19. Sanders, C., and Smillie, L. B. (1984) *Can. J. Biochem. Cell Biol. 62*, 443−448.
20. Graceffa, P. (1989) *Biochemistry 28*, 1282−1287.
21. Lehrer, S. S., and Morris, E. P. (1984) *J. Biol. Chem. 259*, 2070−2072.
22. Pittenger, M. F., Kazzaz, J. A., and Helfman, D. M. (1994) *Curr. Opin. Cell Biol. 6*, 96−104.
23. Spudich, J., and Watt, S. (1971) *J. Biol. Chem. 246*, 4866−4871.
24. Lehrer, S. S., and Kerwar, G. (1972) *Biochemistry 11*, 1211−1217.
25. Ishii, Y., and Lehrer, S. S. (1990) *Biochemistry 29*, 1160.
26. Lehrer, S. S. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 3377−3381.
27. Sanders, C., Burtnick, L. D., and Smillie, L. B. (1986) *J. Biol. Chem. 261*, 12774−12778.
28. Ruiz-Opazo, N., and Nadal-Ginard, B. (1987) *J. Biol. Chem. 262*, 4755−4765.
29. Lehrer, S. S., and Stafford, W. F. (1991) *Biochemistry 30*, 5682−5688.
30. Weeds, A. G., and Pope, B. (1977) *J. Mol. Biol. 111*, 129−157.
31. White, H. D. (1982) *Methods. Enzymol. 85*, 698−709.
32. Williams, D. L., Greene, L. E., and Eisenberg, E. (1984) *Biochemistry 23*, 4150−4155.
33. Ooi, T., Mihashi, K., and Kobayashi, H. (1962) *Arch. Biochem. Biophys. 95*, 1−11.
34. Ebashi, S., and Kodama, A. (1965) *J. Biochem. (Tokyo) 58*, 107.
35. Heeley, D. H., Golosinska, K., and Smillie, L. B. (1987) *J. Biol. Chem. 262*, 9971−9978.
36. White, S. P., Cohen, C., and Phillips, G. N. (1987) *Nature 325*, 826−828.
37. Cho, Y.-J., Liu, J., and Hitchcock-DeGregori, S. E. (1990) *J. Biol. Chem. 265*, 538−545.
38. Montiero, P. B., Lataro, R. k., Ferro, J. A., and Reinach, F. C. (1994) *J. Biol. Chem. 263*, 10461−10466.
39. Flicker, P., Phillips, G. N. J., and Cohen, C. (1982) *J. Mol. Biol. 162*, 495−501.
40. Lorenz, M., Poole, K. J. V., Popp, D., Rosenbaum, G., and Holmes, K. C. (1995) *J. Mol. Biol. 246*, 108−119.
41. Holmes, K. C. (1995) *Biophys. J. 68*, 2s−5s.
42. Williams, D. L., Greene, L. E., and Eisenberg, E. (1988) *Biochemistry 27*, 6987−6983.

BI971568W