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Effects of Troponin on Thermal Unfolding of Actin-Bound Tropomyosin

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Abstract—Differential scanning calorimetry (DSC) was used to study the effect of troponin (Tn) and its isolated components on the thermal unfolding of skeletal muscle tropomyosin (Tm) bound to F-actin. It is shown that in the absence of actin the thermal unfolding of Tm is expressed in two well-distinguished thermal transitions with maxima at 42.8 and 53.8°C. Interaction with F-actin affects the character of thermal unfolding of Tm leading to appearance of a new Tm transition with maximum at about 48°C, but it has no influence on the thermal denaturation of F-actin stabilized by aluminum fluoride, which occurs within the temperature region above 70°C. Addition of troponin leads to significant increase in the cooperativity and enthalpy of the thermal transition of the actin-bound Tm. The most pronounced effect of Tn was observed in the absence of calcium. To elucidate how troponin complex affects the properties of Tm, we studied the influence of its isolated components, troponin I (TnI) and troponin T (TnT), on the thermal unfolding of actin-bound Tm. Isolated TnT and TnI do not demonstrate cooperative thermal transitions on heating up to 100°C. However, addition of TnI, and especially of TnT, to the F-actin–Tm complex significantly increased the cooperativity of the thermal unfolding of actin-bound tropomyosin.

Key words: differential scanning calorimetry, actin, tropomyosin, troponin complex, troponin T, troponin I

The molecular mechanism of muscle contraction is based on a cyclic interaction of myosin heads with actin filaments [1, 2]. In skeletal and cardiac muscles this interaction is regulated by proteins associated with actin filaments—tropomyosin (Tm) and troponin (Tn). Troponin is a complex composed of three components: troponin I (TnI), troponin T (TnT), and troponin C (TnC). The native Tm molecule consists of two polypeptide chains, each with molecular weight of about 33,000. These Tm subunits form so-called "coiled-coil", in which two polypeptide chains are in register. Tm dimers interact with each other in a head-to-tail manner and form a continuous polymer lying in a groove of the actin filament. The results of structural studies suggest that Tm can occupy different positions on the surface of actin filament depending on the absence and presence of myosin, troponin, and calcium [3]. From a comparison of these results with those of kinetic studies, a theoretical model of interaction between F-actin, Tm, and Tn, the so-called "three state model", was proposed in 1993 [4, 5]. The main idea of this model is that the structural unit, consisting of seven actin monomers, tropomyosin dimer, and troponin heterotrimer, is in a dynamic equilibrium between three different states reflecting distinct position of the Tm molecule on the surface of the thin filament. It has been recently decided to name these states as "blocked", "closed", and "open" [3, 4]. The "closed" state corresponds to the interaction of Tm with F-actin in the absence of other proteins, the "blocked" state (a complete blocking of interaction between myosin and actin) takes place only in the presence of troponin and absence of calcium, and the "open" state is realized only in the presence of myosin. Thus, the movements of actin-bound Tm from one distinct position to another are of special importance in regulation of muscle contraction. Therefore, the structural transitions of Tm between three different states are recently the object of detailed studies using various approaches.

Abbreviations: DSC) differential scanning calorimetry; S1) myosin subfragment 1; Tm) tropomyosin; Tn) troponin complex; TnC) troponin C; TnI) troponin I; TnT) troponin T.

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One of these approaches is differential scanning calorimetry (DSC), which has been successfully used for studying complexes of smooth muscle tropomyosin with actin [6, 7]. It was shown that interaction with actin noticeably increased the thermal stability of smooth muscle Tm (the maximum of thermal transition shifted by 3-6°C to higher temperature depending on the Tm/F-actin molar ratio), but it had no effect on the thermal unfolding of F-actin [6]. These data suggest that in the "closed" state (i.e., in the complexes of Tm with F-actin obtained in the absence of other proteins) interaction of Tm with F-actin noticeably changes the character of the thermal unfolding of Tm.

The regulated thin filament, i.e., the complex of actin with Tm and other regulatory proteins (troponin, caldesmon, or calponin), is of particular interest for study by the DSC approach. It would allow the description of another structural state of Tm, the so-called the "blocked" state. Studies in this direction have not been done before. Therefore, in the present work we studied a model system consisting of skeletal muscle proteins: actin, tropomyosin, and troponin. Skeletal Tm exists as two isoforms—α and β [8, 9]. In muscle these chains are present in the ratio of (3-4) : 1, and they can form all three possible dimers: αα, ββ, and αβ. Under physiological conditions, skeletal Tm is a mixture of αα-homodimers and αβ-heterodimers. Thus, it is evident that skeletal Tm is a more complex object than smooth muscle Tm represented by only heterodimers. The thermal unfolding of skeletal muscle Tm has been investigated by DSC in detail [10-12], but its complexes with F-actin were not studied earlier.

Troponin consists of three components: TnI inhibits the ATPase activity of actomyosin, TnT is responsible for troponin binding to Tm, and TnC binds calcium and regulates the interaction of two other components of the troponin complex with actin and tropomyosin. In the absence of Ca^{2+}, TnC interacts weakly with the other two troponin components, while TnI and TnT bind strongly to Tm and fix it in the "blocked" state on the actin filament. An increase in calcium concentration strengthens the interaction between components of the troponin complex and weakens their interaction with Tm [13, 14]. Due to this ability, troponin can participate in the regulation of muscle contraction. The DSC approach was used earlier for studying the thermal unfolding of isolated components of the troponin complex. It was shown that both TnI and TnT demonstrated no cooperative thermal transitions during heating up to 100°C [15]. Troponin C is the only component that denatures with a cooperative transition [16, 17].

In the present work, we applied DSC for the first time to study the complexes of skeletal muscle Tm with F-actin in the presence and absence of troponin or its isolated components.

MATERIALS AND METHODS

Actin preparation. Actin was prepared from rabbit skeletal muscle and purified by two cycles of polymerization–depolymerization [18]. One hour before experiments, G-actin was polymerized by addition of $MgCl_2$ to the final concentration 5 mM and used as F-actin. G-Actin concentration was determined spectrophotometrically using $A^{1\%}_{290nm} = 6.3$ cm^{-1}. Molar concentration of G-actin was calculated using the molecular mass of 42 kD for actin monomers.

Tropomyosin and troponin. Tm, troponin, and components of the troponin complex were prepared from rabbit skeletal muscle as described earlier [19]. Before experiments, lyophilized proteins were dissolved in 10 mM imidazole, pH 7.0, containing 100 mM KCl, 3 mM $MgCl_2$, 1 mM DTT, and 0.1 mM EGTA, centrifuged for 5 min at 14,000*g*, and dialyzed overnight against 100 volumes of the same buffer. Concentrations of Tm and troponin were determined spectrophotometrically using $A^{1\%}_{276nm} = 3.1$ cm^{-1} and $A^{1\%}_{280nm} = 4.7$ cm^{-1}, respectively.

Troponin T and troponin I. Lyophilized proteins were dissolved in 1 mM HCl and dialyzed for at least 3 h against 100 volumes of 30 mM Hepes-KOH, pH 7.3, containing 1 mM $MgCl_2$, 200 mM KCl, and 1 mM β-mercaptoethanol. Concentrations were determined spectrophotometrically using $A^{1\%}_{280nm} = 5.9$ cm^{-1} for TnI and $A^{1\%}_{280nm} = 5.0$ cm^{-1} for TnT.

The purity of the protein preparations was checked by polyacrylamide 8-20% gradient gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) [20].

Actomyosin ATPase activity. The actin-activated Mg^{2+}-ATPase activity of myosin was determined in buffer containing 20 mM imidazole-HCl, pH 7.0, 3 mM $MgCl_2$, and 100 mM KCl. Samples (0.5 ml) contained 25 μM F-actin, 10 μM Tm (and sometimes 10 μM Tn), 0.35 μM myosin subfragment 1 (20 μg), and either EGTA (1 mM) or $CaCl_2$ (0.5 mM). The reaction was initiated by addition of ATP to the final concentration of 1 mM. The ATPase activity was determined after 10 min at 25°C. In preliminary experiments, it was shown that within this time range the accumulation of P_i was linearly dependent on the protein concentration and the time of incubation. The reaction was stopped by addition of $HClO_4$, and the concentration of free P_i in samples was determined [21].

Differential scanning calorimetry. Calorimetric measurements were performed on the DASM-4M differential adiabatic scanning microcalorimeter (Institute for Biological Instrumentation, Pushchino, Russia) with 0.47 ml capillary platinum cells [22]. All measurements were carried out at a heating rate of 1°C/min within a temperature range from 15 to 100°C. An excess pressure of 2-2.5 atm was always kept in the cells to prevent any degassing of solutions during heating. The calibration power W was 25 μW. Instrumental base line was determined at the required heating rate with operating and

control cells filled with buffer used in experiment (30 mM Hepes-KOH, pH 7.3, containing 1 mM $MgCl_2$, 200 mM KCl, and 1 mM β-mercaptoethanol). After cooling the cells to 5°C the buffer was removed from the operating cell, and the cell was filled with protein solution in the same buffer. The reversibility of the thermal denaturation of proteins under investigation was checked by reheating of the sample immediately after cooling from the previous scan. Thermal denaturation of Tm was reversible, actin and troponin melted irreversibly, while isolated TnI and TnT did not demonstrate any cooperative transitions within the temperature range studied (up to 100°C). The heat sorption curves of the studied proteins were analyzed and plotted using the Origin software package (Microcal Inc., USA).

Actin stabilization. To achieve a better separation of transitions of Tm, Tn, and F-actin on thermograms, actin filaments were stabilized with aluminum fluoride, which significantly increases the thermal stability of F-actin [23]. The stabilization occurs in two stages. At first NaF was added to F-actin to the final concentration of 5 mM, and protein was incubated for 5 min at room temperature. Then, $AlCl_3$ was added to the final concentration of 0.5 mM, and F-actin was incubated for 10 min at room temperature [23].

RESULTS AND DISCUSSION

Two well-distinguished thermal transitions were revealed during the thermal denaturation of skeletal muscle Tm (Fig. 1). This agrees well with data of the literature [12]. According to these data, the low-temperature transition (with maximum at 42.8°C) reflects the denaturation of the C-terminal part of Tm molecule with reduced SH-group of Cys190, whereas the high-temperature transition (with maximum at 53.8°C) corresponds to unfolding of the N-terminal part of Tm. The oxidizing of SH-groups with formation of disulfide bond between two Cys190 belonging to two chains of the Tm molecule increases the thermal stability of the C-terminal part of tropomyosin. As a result, thermal transition of the C-terminal part nearly coincides with the thermal transition of the N-terminal part of Tm molecule [12]. During the reheating of a Tm sample the intensity of the low-temperature transition increases, while that of the high-temperature transition decreases (Fig. 1). We suppose that during the first heating of Tm in the presence of β-mercaptoethanol the SH-groups of Cys190 become reduced, and therefore during reheating the C-terminal part of Tm melts at lower temperature. To test this assumption we investigated the dependence of the thermal denaturation of skeletal muscle Tm on the concentration of β-mercaptoethanol (in the range up to 1 mM, i.e., at the maximal concentration that is acceptable in calorimetric experiments). It was shown that decrease in β-mercaptoethanol concentration leads to smaller increase in the intensity of the low-temperature transition of Tm during rescanning (data not shown).

The character of the thermal denaturation of Tm was noticeably changed if it was bound to actin (Fig. 2a). This is reflected in the appearance of a new thermal transition with maximum at 48°C (Fig. 2b). Similar to the data obtained with smooth muscle Tm [6], interaction of skeletal muscle Tm with actin had no effect on the thermal denaturation of F-actin stabilized by aluminum fluoride (Fig. 2a). After heating of the complex Tm–F-actin to 90°C (i.e., after complete irreversible denaturation of actin) and following cooling, only peaks at 42.8 and 52.8°C corresponding to the thermal denaturation of free Tm were observed on heat sorption curve during the reheating. Thus, we conclude that the appearance of the peak at 48°C in the presence of F-actin reflects the thermal denaturation of actin-bound Tm.

Prior to calorimetric studies on regulated actin filament, we performed preliminary experiments directed to

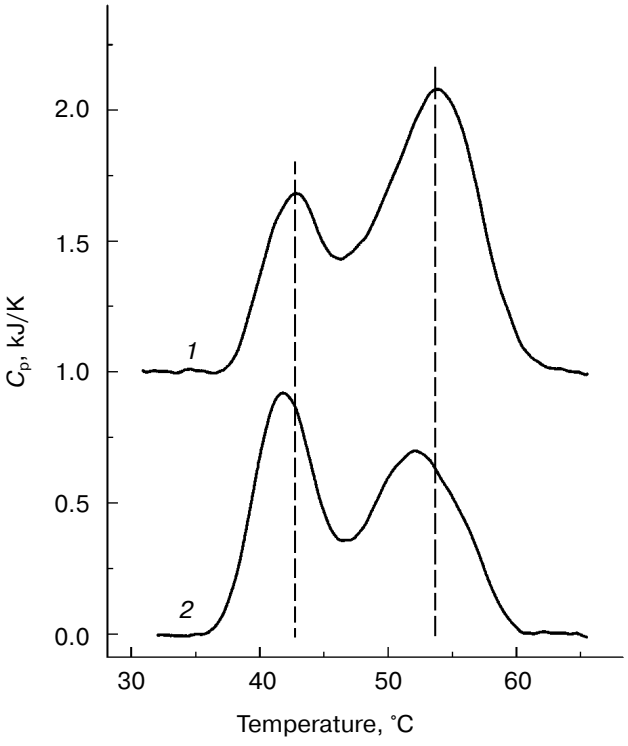

Fig. 1. Temperature dependence of the excess heat capacity (C_p) of skeletal muscle tropomyosin (Tm): first (*1*) and second heating (*2*). Conditions: 35.8 μM Tm, 30 mM Hepes-KOH, pH 7.3, 2 mM $MgCl_2$, 200 mM KCl, and 1 mM β-mercaptoethanol. Dashed lines indicate the positions of the low-temperature and high-temperature transitions of Tm during the first heating.

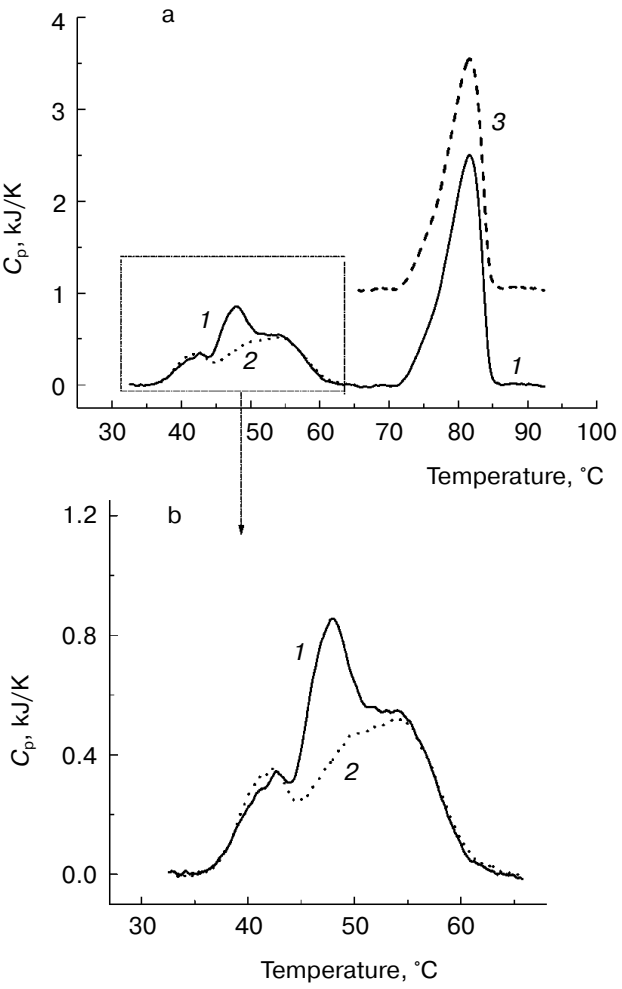

Fig. 2. Temperature dependence of excess heat capacity of the complex of skeletal muscle Tm with F-actin (a) and denaturation region of Tm in the presence (*1*) and absence of F-actin (*2*) (b). For comparison, the thermal transitions of free Tm (16 μM) (*2*) and F-actin (24 μM) (*3*) stabilized by aluminum fluoride are also shown.

study of the functional activity of the complex composed of actin, tropomyosin, and troponin. For this purpose, we analyzed the effectiveness of regulation of actomyosin ATPase by the troponin–tropomyosin complex. To determine the calcium sensitivity of the reconstituted complex, we measured the actomyosin ATPase activity in the presence ($ATPase_{+Ca}$) and absence ($ATPase_{-Ca}$) of calcium. The calcium sensitivity of regulated actomyosin complex calculated by equation:

$$Ca_{sensitivity} = 1 - (ATPase_{-Ca}/ATPase_{+Ca}),$$

was 0.78, which is comparable with data of the literature [24] and indicates formation of regulated actin filament.

After obtaining regulated actin filament, we started the calorimetric studies on the actin complexes with regulatory proteins (Fig. 3).

Only one peak is revealed on the thermograms of the troponin complex (Fig. 3). In the presence of Ca^{2+} the

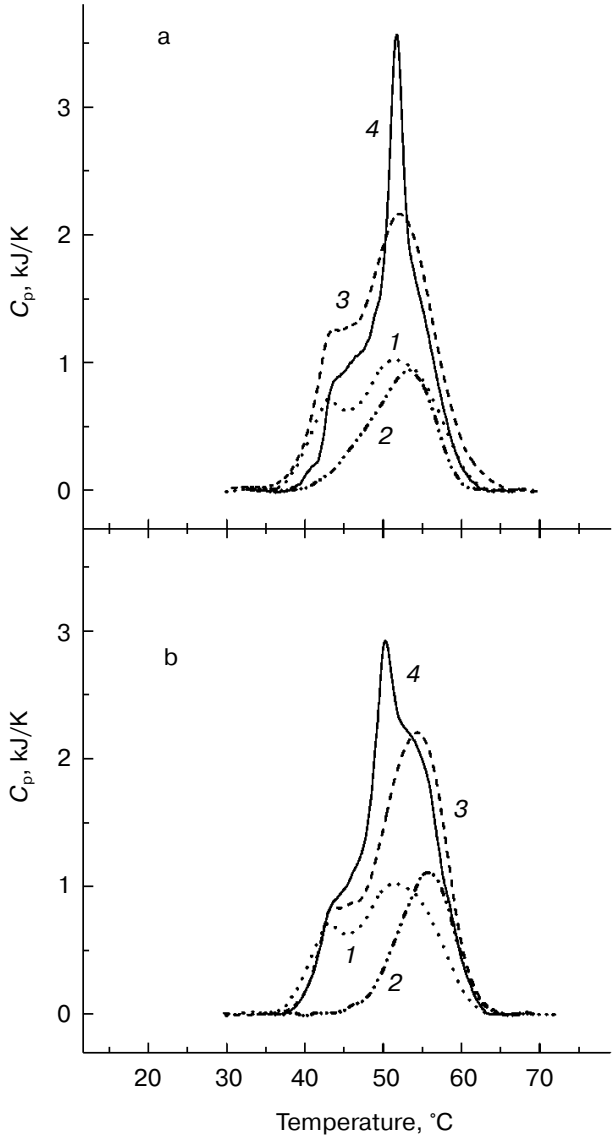

Fig. 3. Effect of troponin (Tn) on the thermal denaturation of skeletal muscle tropomyosin (Tm) bound to F-actin in the presence of 1 mM EGTA (a) or 0.5 mM Ca^{2+} (b). Protein concentration: F-actin, 35 μM; Tm, 25 μM; and Tn, 25 μM. The DSC curves of Tm (*1*), Tn (*2*), and their complex (Tm–Tn) obtained in the absence (*3*) or in the presence of F-actin (*4*) are shown for comparison. In this figure and in Figs. 6 and 7 a temperature region above 70°C corresponding to the thermal denaturation of F-actin stabilized by aluminum fluoride is not shown.

maximum of this peak shifts by 2.6°C to higher temperature (from 53.2 to 55.8°C), and the cooperativity of the thermal transition increases noticeably. These changes, most likely, reflect the fact that the interactions between the components of troponin complex become tighter in the presence of Ca^{2+} [13, 14].

As expected, the thermal denaturation of Tm was independent of Ca^{2+} (compare Figs. 3a and 3b). Addition of calcium to the tropomyosin–troponin complex shifted the maximum of the high-temperature transition by 2.4°C to higher temperature. This shift was completely determined by the changes in the thermal denaturation of Tn. Detailed analysis of the thermograms of Tm, Tn, and their complexes led to the conclusion that the heat sorption curve of the Tm–Tn complex, both in the absence and presence of Ca^{2+}, represents a sum of the thermograms of isolated Tm and Tn. Thus, formation of troponin–tropomyosin complex does not lead to any noticeable changes in the thermal denaturation of its components.

Addition of actin significantly changes the thermal denaturation of troponin–tropomyosin complex (Fig. 3). First, these changes are expressed in significant increase in cooperativity of the high-temperature transition (the peak becomes much higher and narrower). This effect is especially pronounced in experiments performed in the absence of calcium (Fig. 3a). In the presence of Ca^{2+}, a distinctive shoulder in the range of 52-54°C is revealed on the thermogram of F-actin–Tm–Tn complex (Fig. 3b); this shoulder probably reflects the thermal denaturation of the actin-free Tm–Tn complex that was present in excess to actin. In the presence of calcium, thermal denaturation of the troponin–tropomyosin complex is observed exactly in this range (52-54°C) (Fig. 3b). This assumption is also corroborated by the fact that the shoulder at 52-54°C was not observed in the absence of calcium, when the maxima of transitions of the Tm–Tn complex measured in the presence and absence of F-actin almost coincide with each other (Fig. 3a).

The data presented in Fig. 3 indicate that addition of troponin to F-actin-bound tropomyosin leads to formation of a new protein complex demonstrating cooperative thermal transition with maximum at 51.7°C in the absence of calcium (Fig. 3a), and at 50.3°C in the presence of Ca^{2+} (Fig. 3b). In the absence of calcium, i.e., under conditions corresponding to the "blocked" state, such complex demonstrates a very pronounced cooperative transition, which most likely reflects the melting of troponin–tropomyosin complex bound to actin. Under the conditions used (i.e., under conditions when actin filament is stabilized by aluminum fluoride) the binding of troponin–tropomyosin complex to F-actin has no effect (independently of calcium concentration) on the thermal denaturation of F-actin. Thus, the data indicate that the DSC approach probes the conformational changes of actin-bound tropomyosin accompanying the transition of regulated actin filament from the "closed" to the "blocked" state [4].

The analysis of the data presented on Fig. 3 is complicated because troponin complex and Tm denature within the same temperature region. To avoid these difficulties and to analyze the mechanism of interaction of Tm with troponin and actin in more detail, we studied the thermal denaturation of actin-bound Tm in the presence of isolated components of the troponin complex. It is known that troponin complex binds to actin and tropomyosin via two of its components, namely troponin I and troponin T [13, 14]. Therefore, we studied the effects of isolated troponin I and troponin T on the thermal denaturation of actin-bound tropomyosin. In preliminary experiments we found that both TnI and TnT, as well as their equimolar complex, do not have any cooperative thermal transitions within the temperature range from 5 to 100°C, which is in good agreement with data of the literature [15]. The reconstruction of a complex consisting of actin, tropomyosin, and troponin I (or troponin T) allows us to obtain actin filament in the "blocked" state. An important advantage of such synthetic complex is that one of three proteins included in this complex (troponin I or troponin T) does not possess its own cooperative thermal transition and therefore it does not impede registration of the thermal denaturation of the other components of this complex protein assembly.

Prior to DSC studies of the effect of TnI and TnT on the thermal denaturation of actin-bound Tm, we ran a number of control experiments where we studied the effects of the troponin components on the thermal denaturation of F-actin in the absence of Tm and on the thermal unfolding of Tm in the absence of F-actin.

Addition of TnI leads to a pronounced changes in the character of the thermal denaturation of F-actin. These changes are expressed in decrease in the enthalpy and cooperativity of the melting of F-actin (Fig. 4a). These data indicate that there is a direct interaction between TnI and F-actin. Indeed, there are several regions in the structure of TnI (residues 96-116 and probably 128-148) providing its interaction with actin [13, 25]. The effect was much weaker if troponin I was added to F-actin stabilized by aluminum fluoride (Fig. 4b). Apparently, stabilization of actin by aluminum fluoride prevents the effect of troponin I on the structure of fibrillar actin.

In the absence of actin, TnT and TnI (added both separately and together) did not noticeably affect the thermal denaturation of Tm (Fig. 5). There was only a small decrease in amplitude of the high-temperature transition of Tm in the presence of TnT (but not TnI) (Fig. 5). As mentioned above, this peak consists of two overlapping thermal transitions which reflect unfolding of the N-terminal part of the Tm molecule and the C-

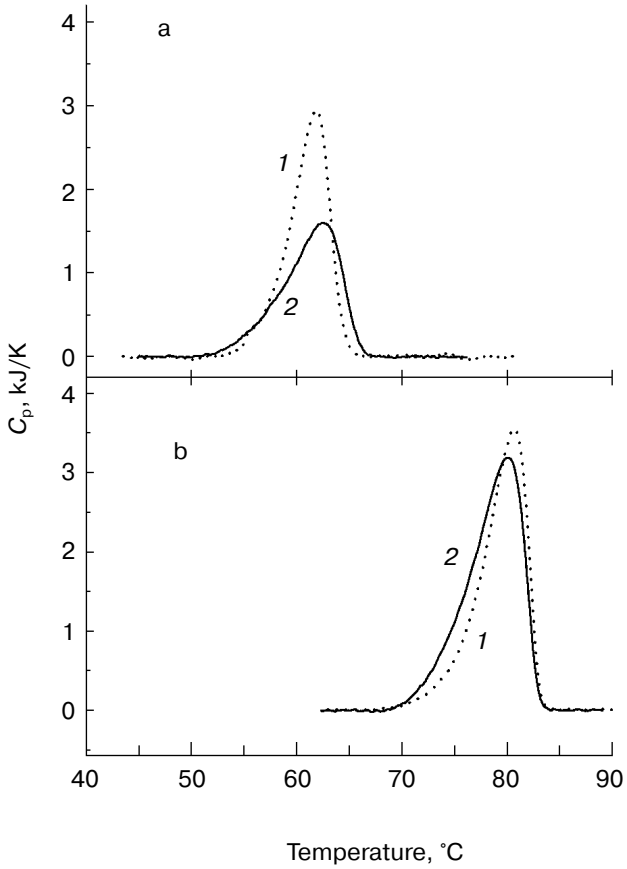

Fig. 4. Influence of troponin I (TnI, 20 μM) on the thermal denaturation of F-actin (30 μM) in the absence of stabilizing factors (a) and in the presence of aluminum fluoride (AlF_4) (b): F-actin without (*1*) and with TnI (*2*).

terminal part of Tm with disulfide bond between two Cys190 belonging to two neighboring Tm monomers. Several sites of Tm are involved in the interaction with TnT [13, 14, 26], and one of these sites is located near Cys190. It seems possible that binding of TnT to this region affects melting of Tm, and this effect is reflected in decrease in the amplitude of the high-temperature transition. Troponin I can interact with tropomyosin even in the absence of actin [13, 27], but this interaction has no effect on the thermal denaturation of tropomyosin, as shown in Fig. 5.

As mentioned above, the thermogram of the complex consisting of tropomyosin and the mixture of TnI and TnT practically does not differ from the thermogram of isolated tropomyosin (see Figs. 5 and 6). However, addition of F-actin to the mixture containing Tm, TnI, and TnT results in the appearance of a sharp cooperative peak with maximum at 49.8°C (Fig. 6). This means that the TnI–TnT complex significantly increases the cooperativity of the thermal transition of actin-bound tropomyosin. It is interesting to note that troponin components affect not only the cooperativity of the thermal transition of actin-bound tropomyosin, but also the maximum temperature of this transition. The maximum of the thermal transition of actin-bound tropomyosin is at 47.9°C (Fig. 7). Addition of TnI increases the amplitude and the cooperativity of the peak of actin-bound tropomyosin, and the maximum of the peak shifts to 48.8°C (Fig. 7). If TnT was added to the actin–tropomyosin complex, even more pronounced increase of the amplitude and cooperativity of the thermal transition of actin-bound Tm was observed, and the thermal transition was at 49.6°C (Fig. 7). A similar effect was observed when the mixture of TnI and TnT was added to the actin–tropomyosin complex (Fig. 7).

In conclusion, interaction with actin changes the character of the thermal denaturation of Tm. If the melt-

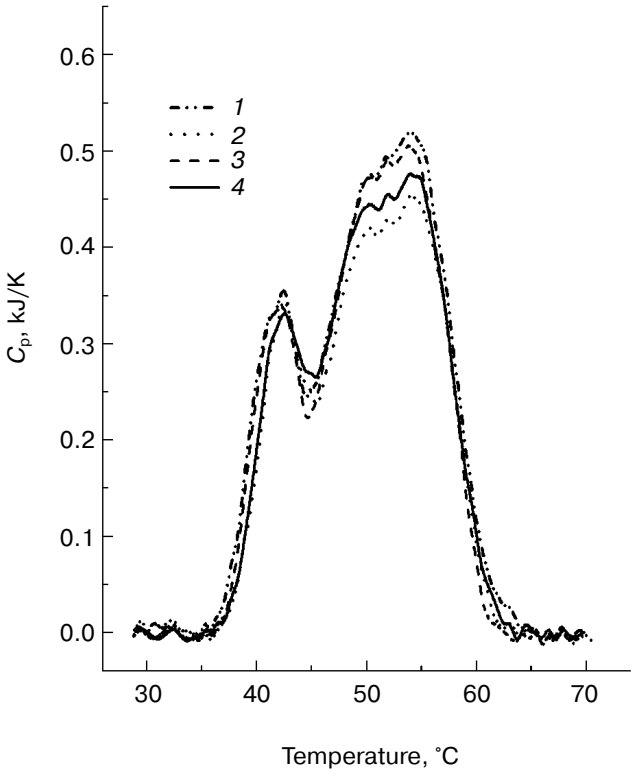

Fig. 5. Temperature dependences of excess heat capacity (DSC curves) of skeletal muscle Tm (*1*) and its complexes with isolated troponin components—troponin T (TnT) (*2*), troponin I (TnI) (*3*), and both TnT and TnI (*4*)—obtained in the absence of actin. Protein concentration: Tm, 16 μM; TnI, 16 μM; TnT, 10 μM.

ing of actin-free tropomyosin is characterized by two thermal transitions with maxima at 42.8 and 53.8°C, then after interaction with actin the additional cooperative peak with maximum between the low- and high-temperature transitions of free tropomyosin appears on the thermogram (Fig. 2). Addition of isolated components of the troponin complex significantly increases cooperativity of this peak, the effect being the most pronounced in the case of TnT and being maximal in the presence of the TnT–TnI complex.

Thus, using the DSC approach we succeeded in studying one of the states of Tm on the actin filament. According to the "three state model", tropomyosin is in the blocking position (i.e., in the "blocked" state) in the complex consisting of actin, tropomyosin, and troponin I (or troponin T). Our data indicate that during transition to this state the cooperativity of the thermal denaturation of actin-bound Tm noticeably increases. Earlier, studying smooth muscle Tm by DSC, we suggested that the cooperativity of its thermal denaturation depends mainly on the strength of the end-to-end interaction of contiguous Tm molecules on the surface of actin filament [6]. If this suggestion is correct, then the increase in the cooperativity of the thermal denaturation of skeletal muscle Tm accompanying its transition to the "blocked" state can be explained by strengthening of such interaction in the presence of separated components of troponin complex, or in the presence of calcium-free troponin.

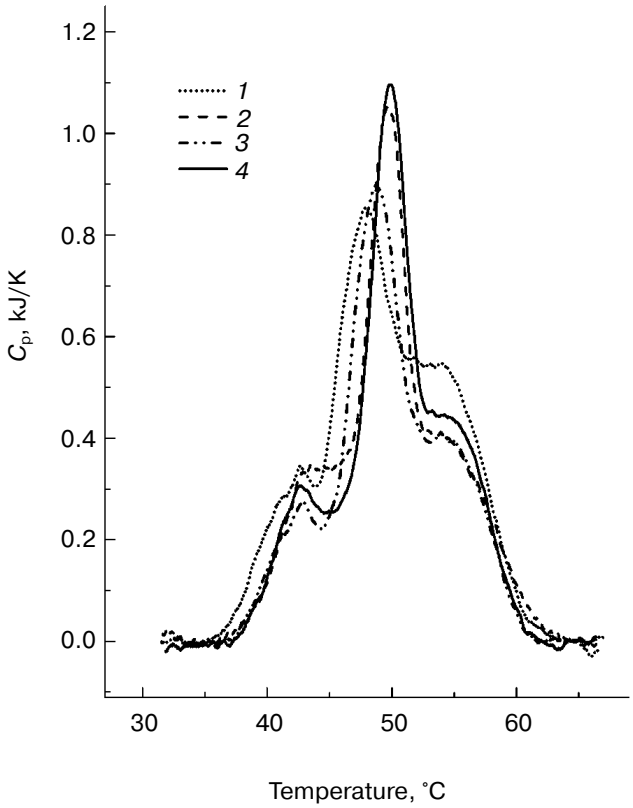

Fig. 7. DSC curves of Tm bound to F-actin in the absence (*1*) and in the presence of TnT (*2*), TnI (*3*), and both TnT and TnI (*4*). Protein concentration: F-actin stabilized by aluminum fluoride (AlF_4), 24 μM; Tm, 16 μM; TnI, 16 μM; TnT, 10 μM. The temperature region above 70°C is not shown.

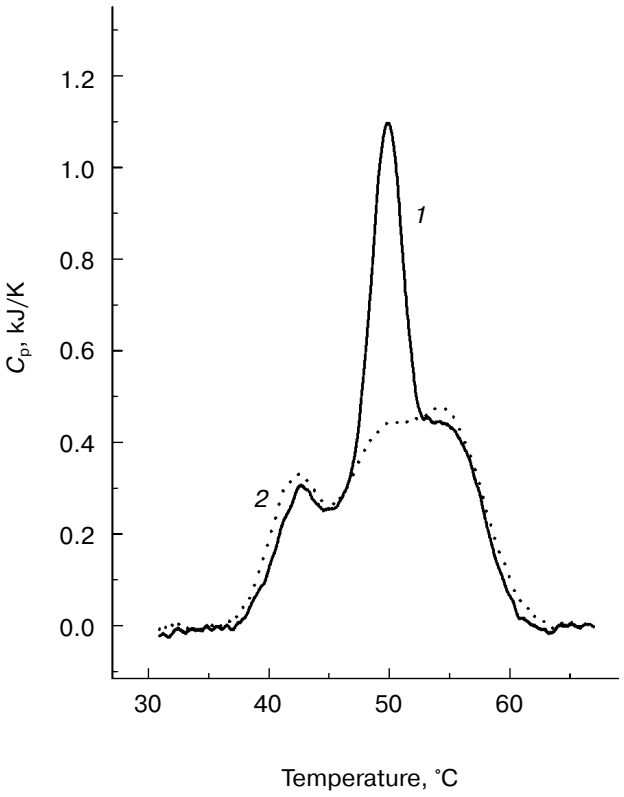

Fig. 6. DSC curves of the Tm–TnT–TnI complex obtained in the presence (*1*) and in the absence (*2*) of F-actin stabilized by aluminum fluoride (AlF_4). Protein concentration: Tm, 16 μM; F-actin, 24 μM; TnT, 10 μM; TnI, 16 μM.

The study of the thermal denaturation of Tm bound to F-actin in the "open" state, i.e., during the interaction of myosin heads with actin, is also of great interest. The results of our preliminary experiments indicate that the transition to this state is accompanied by significant increase in the thermal stability of actin-bound Tm: the maximum of its thermal transition shifts by 5-6°C to higher temperature without appreciable changes in the cooperativity of the transition (E. V. Kremneva, O. P. Nikolaeva, and D. I. Levitsky, unpublished data). However, this will be the subject of a separate publication.

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REFERENCES

1. Poglazov, B. F., and Levitsky, D. I. (1982) *Myosin and Biological Motility* (Kretovich, V. L., ed.) [in Russian], Nauka, Moscow.
2. Levitsky, D. I. (1987) in *Structure and Function of Proteins from Contraction System* (Pinaev, G. P., ed.) [in Russian], Nauka, Leningrad, pp. 5-31.
3. Vibert, P., Craig, R., and Lehman, W. (1997) *J. Mol. Biol.*, **266**, 8-14.
4. McKillop, D. F., and Geeves, M. A. (1993) *Biophys. J.*, **65**, 693-701.
5. Holmes, K. C. (1995) *Biophys. J.*, **68**, 2s-7s.
6. Levitsky, D. I., Rostkova, E. V., Orlov, V. N., Nikolaeva, O. P., Moiseeva, L. N., Teplova, M. V., and Gusev, N. B. (2000) *Eur. J. Biochem.*, **267**, 1869-1877.
7. Levitsky, D. I., Nikolaeva, O. P., Orlov, V. N., Pavlov, D. A., Ponomarev, M. A., and Rostkova, E. V. (1998) *Biochemistry (Moscow)*, **63**, 322-333.
8. Mak, A. S., Smillie, L. B., and Stewart, G. R. (1980) *J. Biol. Chem.*, **255**, 3647-3655.
9. Perry, S. V. (2001) *J. Muscle Res. Cell Motil.*, **22**, 5-49.
10. Potekhin, S. A., and Privalov, P. L. (1982) *J. Mol. Biol.*, **159**, 519-535.
11. Sturtevant, J. M., Holtzer, M. E., and Holtzer, A. (1991) *Biopolymers*, **31**, 489-495.
12. Williams, D. L. J., and Swenson, C. A. (1981) *Biochemistry*, **20**, 3856-3864.
13. Filatov, V. L., Katruha, A. G., Bulargina, T. V., and Gusev, N. B. (1999) *Biochemistry (Moscow)*, **64**, 969-985.
14. Farah, C. S., and Reinach, F. C. (1995) *FASEB J.*, **9**, 755-767.
15. Morozova, L. A., Gusev, N. B., Shnyrov, V. L., and Permyakov, E. A. (1988) *Biokhimiya*, **53**, 531-540.
16. Ingraham, R. H., and Swenson, C. A. (1983) *Eur. J. Biochem.*, **132**, 85-88.
17. Tsalkova, T. N., and Privalov, P. L. (1980) *Biochim. Biophys. Acta*, **624**, 196-204.
18. Spudich, J. A., and Watt, S. (1971) *J. Biol. Chem.*, **246**, 4866-4871.
19. Gusev, N. B., and Dobrovol'skii, A. B. (1978) in *Biophysical and Biochemical Methods of Analysis of Muscle Proteins* (Ivanitskii, G. R., ed.) [in Russian], Nauka, Leningrad, pp. 151-165.
20. Laemmli, U. K. (1970) *Nature*, **227**, 680-685.
21. Panusz, H. T., Graczyk, G., Wilmanska, D., and Skarzynski, J. (1970) *Analyt. Biochem.*, **35**, 494-504.
22. Privalov, P. L., and Potekhin, S. A. (1986) *Meth. Enzymol.*, **131**, 4-51.
23. Nikolaeva, O. P., Dedova, I. V., Khvorova, I. S., and Levitsky, D. I. (1994) *FEBS Lett.*, **351**, 15-18.
24. Potter, J. D. (1982) *Meth. Enzymol.*, **85**, 241-264.
25. Perry, S. V. (1999) *Mol. Cell Biochem.*, **190**, 9-32.
26. Ogut, O., and Jin, J.-P. (2000) *J. Biol. Chem.*, **275**, 26089-26095.
27. Pearlstone, J. R., and Smillie, L. S. (1983) *J. Biol. Chem.*, **258**, 2534-2542.