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Muscle-Type Tropomyosin of Sea Urchin Egg Increases the Actin-Binding of Nonmuscle-Type Tropomyosin

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Tropomyosin isoforms in eggs of several species of sea urchins are classified into two types, muscle-type and nonmuscle-type, based on their antigenicities. Their actin-binding abilities were investigated using muscle-type isoform (32K) and nonmuscle-type isoform (30K), which were purified by the method previously reported and separated by isoelectric focusing from eggs of sea urchin, *Strongylocentrotus intermedius*. Co-sedimentation assays revealed that 32K could stoichiometrically bind to actin filaments independently of the 30K, but 30K alone bound very poorly. The actin-binding of 30K was, however, considerably increased in the presence of 32K, and the molar ratio of the bound 30K and 32K was approximately 1:1. The increase in the actin-binding of 30K is probably caused by the interaction of 30K with 32K in a head-to-tail manner, as indicated by the higher specific viscosity of the mixture than that of 32K alone.

Key words: actin-binding ability, head-to-tail interaction, muscle-type isoform, nonmuscle-type isoform, sea urchin egg tropomyosin.

As echinoderm eggs and embryos show profound actin-based morphological changes, such as formation of the fertilization corn, elongation of microvilli, formation of the contractile ring, ingression of mesenchyme cells, and gastrulation (*1-5*), they are suitable materials to investigate the microfilament organization. In echinoderm eggs there are many actin regulatory proteins such as fascin (*6, 7*), profilin (*7*), depactin (*8*), α-actinin (*9*), gelsolin-like protein (*10*), fodrin (*11*), and spectrin (*12*). Previously, we have identified tropomyosin in sea urchin eggs (*13, 14*), and shown its localization to be at the cortex and cleavage furrow in the fertilized egg (*15, 16*).

Tropomyosin is an integral component of the actin-based contractile apparatus and cytoskeleton of muscle and nonmuscle cells. In striated muscle, tropomyosin together with troponin complex confers Ca^{2+}-dependent regulation on the actin-myosin interaction, whereas the same function as in striated muscle is not expected in smooth muscle, and instead the association of tropomyosin with caldesmon is believed to be involved in the regulation of thin filament-linked contraction. In nonmuscle cells, multiple tropomyosin isoforms are expressed by transcription from multiple genes and by specific alternative splicing of the transcripts (*17-20, 24*). As these isoforms are involved in actin-based cytoskeletal architecture, such as the ruffling membrane, stress fibers, and the contractile ring (*15, 21, 22*), they are believed to be responsible for the regulation of cell motility and shape. Five to seven isoforms of tropomyosin in mammalian and avian cultured nonmuscle cells are classified into two groups with high and low molecular weights (*23, 24*). Furthermore, functional differences between isoforms have been suggested by the observation that high-molecular-weight isoforms more effectively protected actin filaments from the severing activities of gelsolin than low-molecular-weight isoforms did. Additionally, the down-regulation of high-molecular-weight isoforms in transformed cells is concurrent with the poorly organized, diminished actin cytoskeleton and rounded cellular morphology characteristic of the transformed phenotype (*20, 25, 26*). However, despite the clear importance of tropomyosin, it is difficult to ascertain the distinct contributions of each isoform to different cellular functions.

Sea urchin eggs also contain multiple isoforms of tropomyosin, of which the molecular weights and pI values are different among four species of the sea urchin, *Hemicentrotus pulcherrimus, Pseudocentrotus depressus, Anthocidaris crassispina*, and *Strongylocentrotus intermedius* (*16*). The egg tropomyosin isoforms of these species, however, can be clearly classified into two types with different antigenicities, *i.e.*, muscle-type isoform with antigenicity common to that of lantern muscle tropomyosin and nonmuscle-type one with antigenicity common to that of *S. intermedius* egg tropomyosin isoform 30K. Since both types of isoforms are present in unfertilized eggs consistently among the four species of the sea urchin, their existence probably does not reflect functional redundancy, but rather some important functional differences between the isoforms that have been conserved through evolution. However, those differences between egg tropomyosin isoforms with muscle- and nonmuscle-type antigenicities are not clear, because so far, distinct localization of the isoforms in the fertilized egg has not been possible by immunofluorescence microscopic observation (*16*), and no change in the isoform composition

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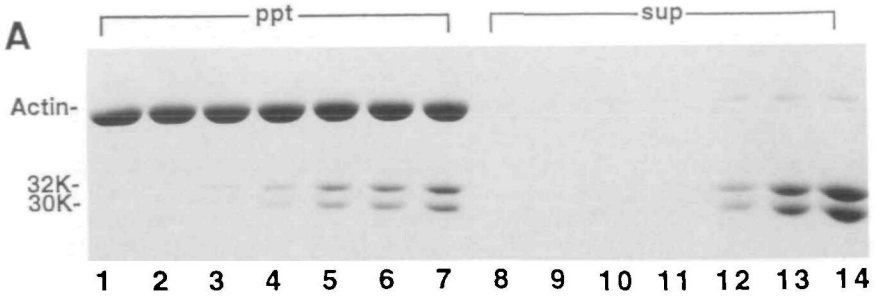
Abbreviations: TM, tropomyosin; TCA, trichloroacetic acid; IEF, isoelectric focusing.



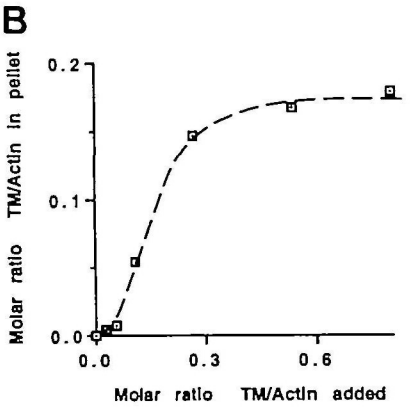


Fig. 2. **Actin-binding abilities of egg tropomyosin isoforms prepared by IEF.** The actin-binding ability of egg tropomyosin prepared by IEF was examined by co-sedimentation. F-Actin was incubated with increasing concentrations of a 1:1 mixture of 32K and 30K prepared by IEF in the binding buffer of 100 mM KCl, 6 mM $MgCl_2$, 0.5 mM β-mercaptoethanol, 0.2 mM EGTA, 0.5 mM ATP, and 5 mM Tris-HCl, pH 7.2. The incubated mixtures were ultracentrifuged. The precipitates (lanes 1-7) and supernatant (lanes 8-14) were subjected to SDS-PAGE using 10% polyacrylamide separating gel (A). The amounts of 32K and 30K bound to actin shown in A (lanes 1-7) were quantified by densitometric scanning and plotted as bound TM/actin *versus* added TM/actin (B). All the reaction mixtures contained 3.7 μM F-actin. 32K or 30K concentrations were: lanes 1 and 8, no TM added; lanes 2 and 9, 0.05 μM; lanes 3 and 10, 0.1 μM; lanes 4 and 11, 0.2 μM; lanes 5 and 12, 0.5 μM; lanes 6 and 13, 1.0 μM; lanes 7 and 14, 1.45 μM.

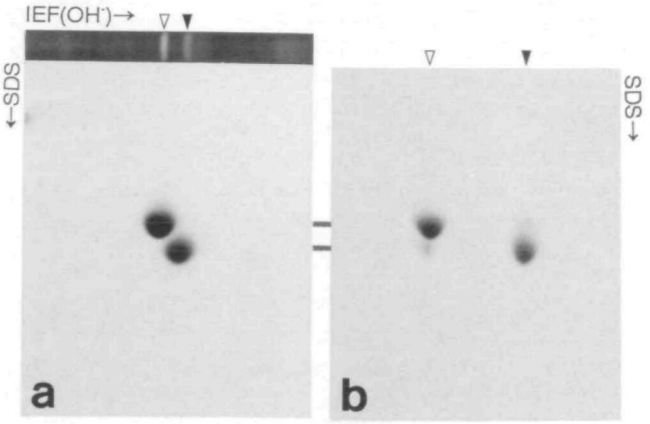


Fig. 1. **Isolation of tropomyosin isoforms by isoelectric focusing.** One hundred microliters of the tropomyosin fraction from the sea urchin egg (100 μg/ml) was subjected to isoelectric focusing on agarose gel. After focusing, the gels were treated with 5% TCA for 15 min to visualize protein bands and subjected to SDS-PAGE (a). Aliquots of 100 μl of the isolated 30K and 32K fractions were subjected to SDS-PAGE by side-by-side (b). White and black arrowheads correspond to 32K and 30K, respectively.

has been observed after fertilization or during early development (*27*). Previously, we have shown that actin-binding ability of nonmuscle-type 30K is slightly lower than that of muscle-type 32K of *S. intermedius* egg tropomyosin, when both isoforms are included in the reaction mixture (*14*).

In this study, we separated the two isoforms by isoelectric focusing and investigated their actin-binding properties in detail. Co-sedimentation assays revealed that 32K alone can bind to actin filaments stoichiometrically, whereas 30K binds very poorly. Interestingly, the actin-binding of 30K is considerably increased by the addition of 32K to the reaction mixture, and the binding ratio of the two isoforms reaches 1:1 in spite of the large difference in actin-binding abilities found in experiments with the single components alone. Based on these results, the distribution of these isoforms on microfilaments is discussed.

MATERIALS AND METHODS

Egg Tropomyosin Fractionation—Eggs of the sea urchin, *S. intermedius*, which was supplied from Akkeshi Marine Biological Station of Hokkaido University, were used in this study. The eggs were collected by intracoelomic stimulation with 0.55 M KCl, then dejellied and washed. An egg tropomyosin fraction was prepared as described previously (*13*).

Preparative Isoelectric Focusing—Isoelectric focusing (IEF) for preparation of the 32K and 30K fractions was based on the method of Hirabayashi (*28*) with some modifications. The egg tropomyosin fraction was dialyzed against a sample solution (7 M urea, 0.1% β-mercaptoethanol), then applied to agarose IEF gel consisting of ampholines (pH 3.5-10 and 4-6), 7 M urea, 15% sorbitol, and 100 mM 2-(*N*-morpholino)ethanesulfonic acid (MES). After focusing at 4°C for 18 h at 500 V, the gel was treated with 5% trichroloacetic acid (TCA) for 15 min to prevent diffusion of proteins out of the gel and to visualize the protein bands. Two bands corresponding to 30K and 32K egg tropomyosin isoforms were cut out with a razor blade. The isoforms were extracted separately with extraction buffer (7 M urea, 1% β-mercaptoethanol, 1 mM Tris-HCl, pH 7.5), and ultracentrifuged at 210,000 × *g* for 30 min at 4°C. Each of the supernatants was concentrated with a Centricon (Amicon), and dialyzed against Tris-HCl buffer (pH 7.5) for 3 h. The dialysate was quickly frozen in liquid nitrogen, dried under vacuum, and stored at −20°C until use.

SDS-PAGE—SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli (*29*) using 10% polyacrylamide separating gel.

Actin-Binding Assay—The actin-binding assay was based on the co-sedimentation method of Eaton *et al.* (*30*). Actin was purified from acetone powder of rabbit skeletal muscle (*31*). A typical reaction mixture contained F-actin (3.7 or 5.8 μM) and various amounts of tropomyosin in binding buffer containing 100 mM KCl, 6 mM $MgCl_2$, 0.5

mM ethyleneglycol bis(2-aminoethylether)-*N,N,N′,N′*-tetraacetic acid (EGTA), 2 mM adenosine triphosphate (ATP), 0.5 mM β-mercaptoethanol, and 10 mM Tris-HCl pH 7.2. The mixture was incubated for 2 h at room temperature and ultracentrifuged at 210,000×*g* for 1 h at 4°C. The supernatant was carefully removed and the pellet rinsed once with 50 μl of binding buffer. The supernatant and pellet were mixed with SDS gel sample buffer and analyzed by SDS-PAGE. The molar ratio of bound tropomyosin to F-actin was estimated by scanning the Coomassie Brilliant Blue (CBB)-stained gel with a BioImage analyzer (Millipore), with chicken skeletal muscle tropomyosin as a standard.

Measurement of Specific Viscosity—The measurement of specific viscosity was based on the falling ball method of Maclean-Fletcher and Pollard (*38*). Each tropomyosin isoform was concentrated and dialyzed against 30 mM KCl, 6 mM $MgCl_2$, 0.1% β-mercaptoethanol, 0.5 mM EGTA, and 10 mM Tris, pH 6.4 for 6 h. The 32K solution (20 μM) was transferred into a capillary tube and the viscosity was measured at intervals at 25°C. Then the 32K solution was taken from the capillary tube and mixed with an equal volume of the 30K solution (20 μM). After incubation of the mixture for 2 h, the viscosity was measured at 25°C.

RESULTS

In the earlier reports, it was shown that tropomyosin isoforms with muscle-type and nonmuscle-type antigenicities from sea urchin eggs could not be separated from each other by hydroxyapatite column chromatography (*14, 32*), but we found definite differences in pI values between the two isoforms of *S. intermedius*, muscle-type 32K and nonmuscle-type 30K (*14*). Taking advantage of the differences, we tried to use isoelectric focusing to separate these isoforms. As shown in Fig. 1a, two protein bands corresponding to 30K and 32K were detected in the TCA-treated agarose gel after isoelectric focusing for 18 h. Two fractions from the respective bands cut from the agarose gel were subjected to SDS-PAGE side-by-side (Fig. 1b). Slight cross-contamination of 30K (2-5%) could not be removed from the 32K fraction, while the 30K fraction contained 30K exclusively. We confirmed that there was no difference in the respective pI values and molecular weights of the

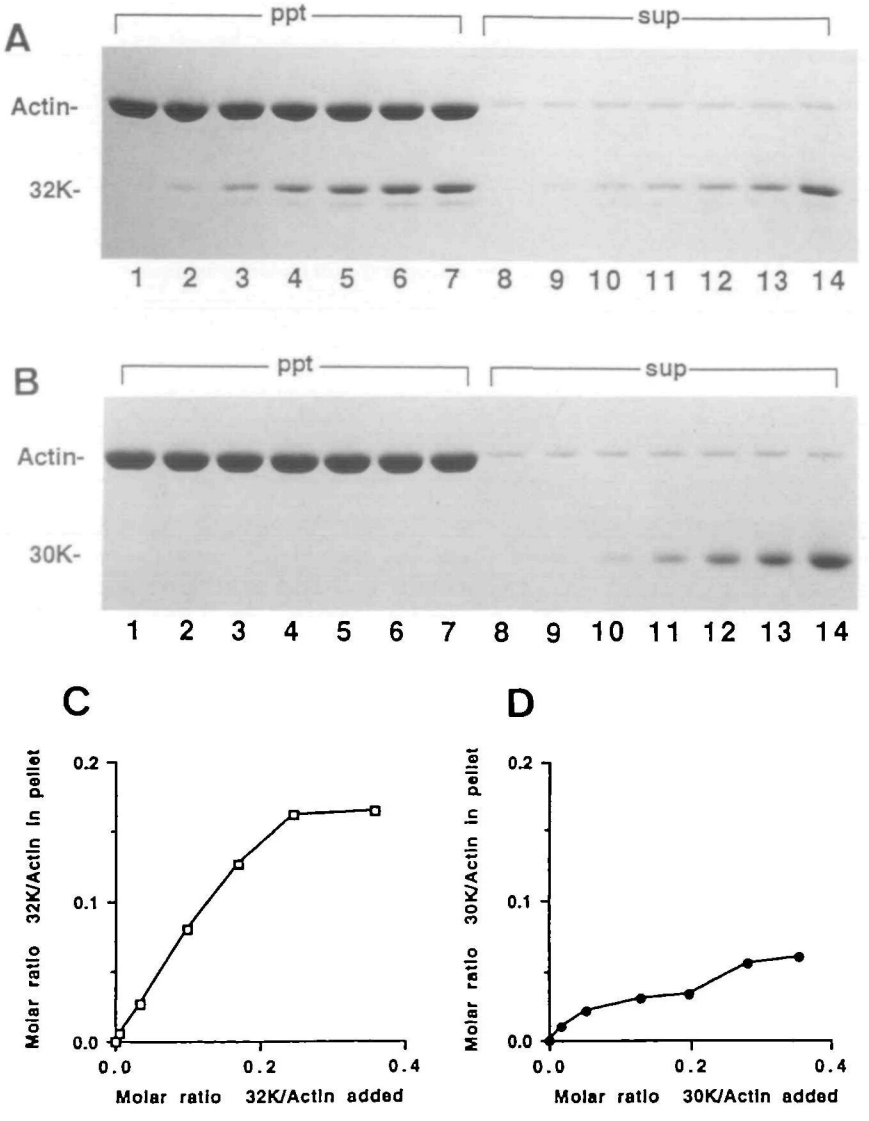
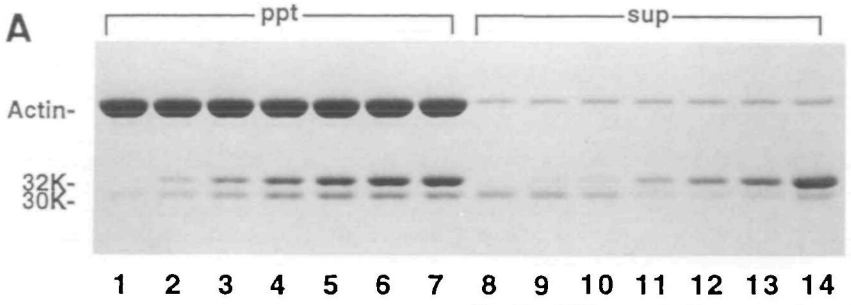


Fig. 3. **Actin-binding abilities of 32K and 30K.** Binding of 32K is shown in A and C, and that of 30K in B and D. F-Actin was incubated with increasing concentrations of 32K or 30K. After ultracentrifugation, the precipitates (lanes 1-7) and supernatant (lanes 8-14) were subjected to SDS-PAGE. Slight cross-contamination of 30K in the 32K fraction could be observed in the precipitate (Fig. 3A, lanes 4-7). The amounts of 32K and 30K bound to actin shown in A and B were quantified by densitometric scanning and the data are plotted as bound TM/actin *versus* added TM/actin in C and D, respectively. All the reaction mixtures contained 5.8 μM F-actin. (A) 32K concentrations were: lanes 1 and 8, no 32K added; lanes 2 and 9, 0.03 μM; lanes 3 and 10, 0.2 μM; lanes 4 and 11, 0.58 μM; lanes 5 and 12, 0.98 μM; lanes 6 and 13, 1.42 μM; lanes 7 and 14, 2.07 μM. (B) 30K concentrations were: lanes 1 and 8, no 30K added; lanes 2 and 9, 0.1 μM; lanes 3 and 10, 0.29 μM; lanes 4 and 11, 0.74 μM; lanes 5 and 12, 1.14 μM; lanes 6 and 13, 1.62 μM; lanes 7 and 14, 2.06 μM.



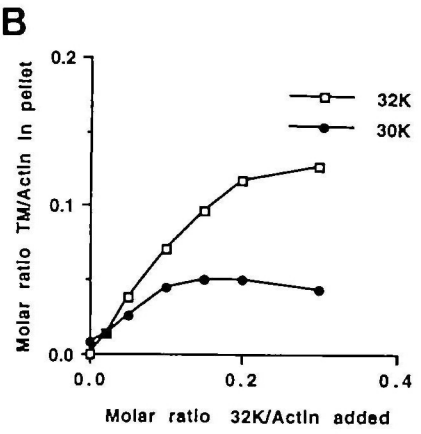


Fig. 4. **Effect of 32K on the binding of 30K to actin.** F-Actin was mixed with 30K and incubated for 2 h, then increasing concentrations of 32K were added to the reaction mixture and the whole was further incubated for 30 min. After ultracentrifugation, the precipitates (lanes 1-7) and the supernatants (lanes 8-14) were subjected to SDS-PAGE (A). The amounts of 32K and 30K bound to actin shown in A were quantified and plotted as bound TM/actin *versus* added TM/actin (B). All the reaction mixtures contained 5.8 μM F-actin. 32K concentrations were: lanes 1 and 8, no 32K added; lanes 2 and 9, 0.12 μM; lanes 3 and 10, 0.29 μM; lanes 4 and 11, 0.58 μM; lanes 5 and 12, 0.87 μM; lanes 6 and 13, 1.16 μM; lanes 7 and 14, 1.74 μM. 30K concentrations were: lanes 1 and 8, 0.25 μM; lanes 2 and 9, 0.28 μM; lanes 3 and 10, 0.33 μM; lanes 4 and 11, 0.39 μM; lanes 5 and 12, 0.44 μM; lanes 6 and 13, 0.46 μM; lanes 7 and 14, 0.47 μM.

32K and 30K without (*14*) and with TCA treatment for preparation. These 32K and 30K fractions were used for the following experiments.

In our earlier study (*14*), the actin-binding ratio of 32K to 30K was close to 1:1 in the sea urchin egg tropomyosin fraction. To confirm the actin-binding abilities of tropomyosin isoforms prepared by isoelectric focusing, we carried out an actin-binding assay using a 1:1 mixture of the 32K and 30K. Figure 2A shows an SDS-PAGE pattern of pellet and supernatant fractions obtained by co-sedimentation of increasing concentrations of tropomyosin with F-actin. By scanning the CBB-stained gel on the BioImage analyzer, a binding curve was obtained, showing the molar ratios of tropomyosin/actin in the pellet *versus* those added, as shown in Fig. 2B. The curve was S-shaped, implying cooperative binding of tropomyosin to F-actin. The binding saturated at 0.16-0.17 molar binding ratio of tropomyosin to F-actin in the pellet, and the values were typical ones between the two proteins. Therefore, no difference was observed in actin-binding properties before (*14*) and after preparation by isoelectric focusing. The actin-binding ratio of 32K to 30K was close to 1:1 in the latter preparation.

To examine the respective binding abilities of 30K and 32K to F-actin, we performed co-sedimentation assays using the 32K and 30K fractions prepared by isoelectric focusing. As shown in Fig. 3 (A and C), 32K bound to F-actin increased depending on its concentration until the binding ratio of 32K/actin reached to 0.17. In this experiment, the actin-binding curve of 32K was not S-shaped, but whether this indicates no cooperative binding of 32K to F-actin is unknown. Unexpectedly, 30K bound to actin very poorly, as shown in Fig. 3 (B and D). The same result was observed in the presence of 15 mM $MgCl_2$ (data not shown). Since the 30K could bind to F-actin as well as 32K when the two isoforms were co-present in the reaction mixture (Fig. 2), this result raised the possibility that the actin-binding property of 30K was significantly altered in the presence of 32K. Furthermore, 30K contaminating the 32K fraction bound to actin together with 32K (Fig. 3A), suggesting that the binding of 30K to F-actin was increased by the presence of 32K.

Thus, to investigate the effect of 32K on the binding of 30K to actin, we carried out co-sedimentation experiments using increasing concentrations of 32K with constant concentrations of 30K and F-actin. As shown in Fig. 4, the binding of 30K to actin was significantly increased as the amount of 32K was increased in the reaction mixture, whereas it was almost undetectable in the absence of 32K. In this experiment, competitive binding of 32K against 30K (Fig. 4A, lane 7 and Fig. 4B) was observed when the ratio of 32K/actin exceeded 0.2. Since the 32K fraction used in the experiments contained slight contamination with 30K (Fig. 1b), we could not rule out the possibility that a part of the increase in the binding of 30K was caused by the increased concentration of 30K in the reaction mixture.

To examine further the relationship between 30K and 32K in actin-binding, increasing amounts of the 30K fraction were added to two constant concentrations of 32K (Fig. 5). At the lower concentration of 32K (0.59 μM) (Fig. 5, A and C), the binding of 30K to F-actin increased until it became close to that of 32K. Although the total binding of 30K plus 32K to actin did not reach saturation, no increase in the binding of 30K was observed upon further addition of the 30K fraction to the reaction mixture. The result indicated that the increase in the 30K binding to F-actin depends on the amount of 32K bound to actin, and raised the possibility that one 32K molecule interacts with one 30K molecule on the binding of the 30K to actin. As shown in Fig. 5A, some 32K seemed to remain in the supernatant, even at a nonsaturating concentration of tropomyosin. One possibility is that some population of the 32K fraction in this experiment lost the binding ability to actin. In the presence of a higher concentration of 32K (1.1 μM), 30K of increasing concentrations seemed to displace 32K which had bound to F-actin in a competitive manner, although 30K alone had a very poor actin-binding ability (Fig. 5, B and D).

The result that 30K could displace 32K competitively until the molar ratio of 30K to 32K reached to 1:1 (Fig. 5D) implied that the interaction of 32K-30K was stronger than

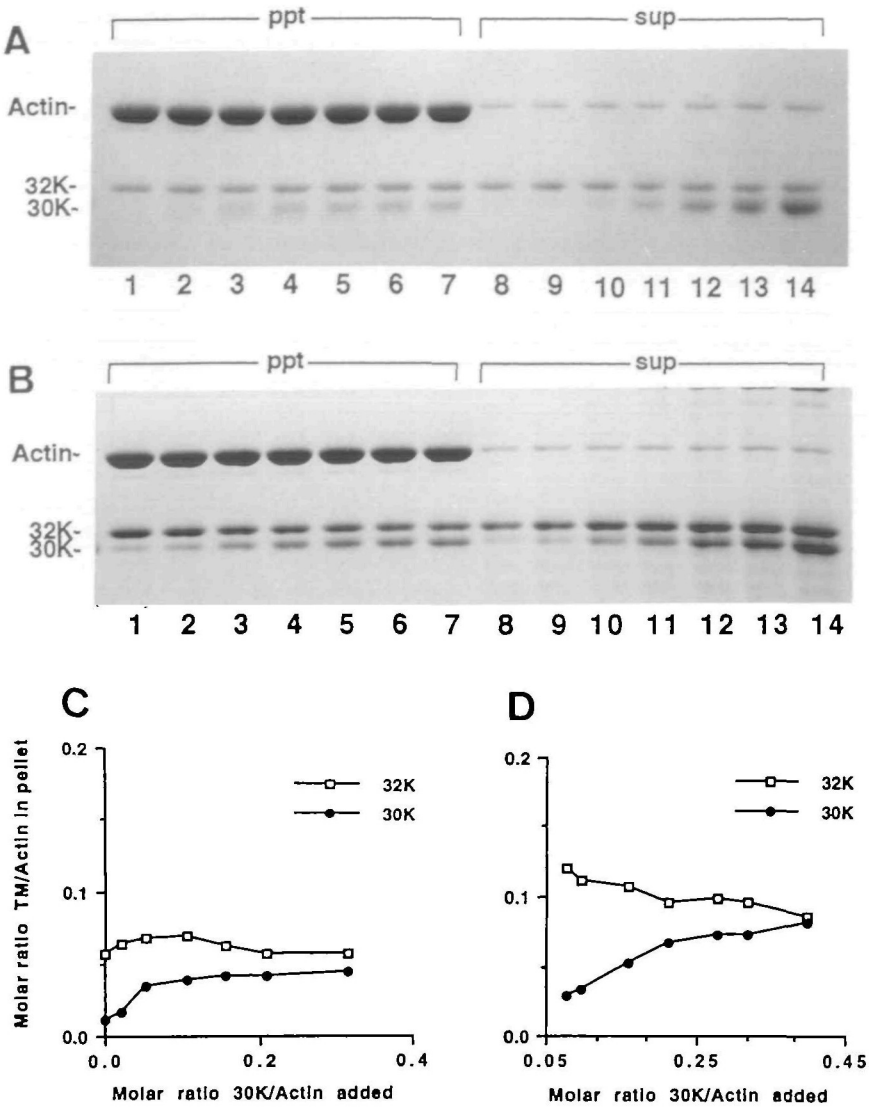


Fig. 5. **Effect of 30K on the binding of 32K to actin.** F-Actin was mixed with 0.58 μM (A and C) or 1.1 μM (B and D) of 32K and the mixture was incubated for 2 h, then increasing concentrations of 30K were added, and the reaction mixture was further incubated for 30 min. All the reaction mixtures contained 5.8 μM F-actin at the final concentration. After ultracentrifugation, the precipitates (lanes 1-7) and the supernatant (lanes 8-14) were subjected to SDS-PAGE (A and B). The amounts of 32K and 30K bound to actin shown in A and B were quantified and plotted as bound TM/actin *versus* added TM/actin in C and D, respectively. (A) 30K concentrations were: lanes 1 and 8, 0.05 μM; lanes 2 and 9, 0.12 μM; lanes 3 and 10, 0.3 μM; lanes 4 and 11, 0.61 μM; lanes 5 and 12, 0.92 μM; lanes 6 and 13, 1.22 μM; lanes 7 and 14, 1.83 μM. (B) 30K concentrations were: lanes 1 and 8, 0.44 μM; lanes 2 and 9, 0.56 μM; lanes 3 and 10, 0.92 μM; lanes 4 and 11, 1.24 μM; lanes 5 and 12, 1.61 μM; lanes 6 and 13, 1.84 μM; lanes 7 and 14, 2.31 μM.

that of 32K-32K. It is known that higher viscosity of muscle tropomyosin solution at low ionic strength is closely related to the extent of linear head-to-tail polymerization of the protein (*17*). Thus, to detect a possible difference between the interactions of 32K-32K and 32K-30K, we measured the viscosities of 32K alone and a mixture of 32K and 30K by the falling ball method at a low ionic strength (30 mM KCl). As shown in Fig. 6, the specific viscosity of the 32K and 30K mixture was slightly higher than that of 32K alone, showing that the interaction of 32K-30K was stronger than that of 32K-32K and that the increase in actin-binding of 30K was probably due to head-to-tail interaction between 32K and 30K. The same result was obtained at a lower ionic strength (5 mM KCl, data not shown).

DISCUSSION

The tropomyosin isoforms in sea urchin eggs from several species have been classified into two types, muscle-type and nonmuscle-type, based on their antigenic properties, although the pI values and molecular weights of the isoforms in each type are different among the sea urchins (*14, 16*).

In this study, we examined actin-binding properties using egg tropomyosin isoforms of the two types, muscle-type 32K and nonmuscle-type 30K, from *S. intermedius*. Although the actin-binding ratio of 32K to 30K was 1:1 at saturation when a mixture of the two isoforms was used for actin-binding assays (Fig. 2), we found that 32K alone could stoichiometrically bind to actin, but 30K alone bound very poorly (Fig. 3), suggesting that the actin-binding property of 30K was significantly influenced by the presence of 32K.

The binding of 30K to actin was increased by the presence of 32K in a concentration (32K)-dependent manner until the content of tropomyosin bound to actin reached the saturation level (Fig. 4, lanes 1-6), and the content of 30K bound to actin never exceeded that of 32K which had bound to actin, even below the saturation level (Fig. 5, A and C), suggesting the possibility that one 32K molecule interacts with one 30K molecule on binding to actin. Although the two isoforms could bind to actin at 1:1 ratio when they were mixed simultaneously with actin for the assay (Fig. 2), the binding ratio of 30K to 32K was

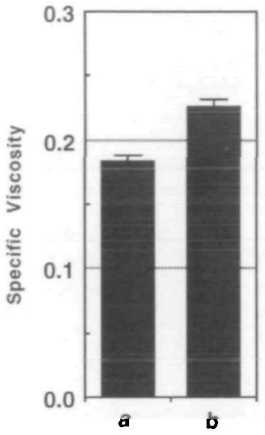


Fig. 6. **Specific viscosity of 32K and a mixture of an equal amount of 32K and 30K.** The 32K and 30K fractions prepared by IEF were concentrated to 20 μM and then dialyzed against 30 mM KCl, 6 mM $MgCl_2$, 0.1% β-mercaptoethanol, 0.5 mM EGTA, and 10 mM Tris, pH 6.4, for 6 h to estimate the specific viscosity. Measurements of the viscosity were performed by the falling ball method at 25°C. The averages and standard deviations from five measurements are shown for 32K (a) and the mixture of the two isoforms (b). Consistency of the protein concentration during the measurement of viscosity was confirmed by the estimation of protein concentration using densitometric scanning of the CBB-stained gel with chicken skeletal muscle tropomyosin as a standard after measurement of viscosity.

slightly lower than 1:1 when 30K was added to pre-formed 32K-actin complex (Fig. 5, A and C). This may be attributable to insufficient duration (30 min) of incubation of 30K with the complex. Since the same result was obtained using egg tropomyosin isoforms with muscle-type and nonmuscle-type antigenicities from *Toxopneustes pileolus* (data not shown), binding of the nonmuscle-type isoform to actin is increased by the muscle-type isoform in other sea urchin eggs as well.

The actin-binding assay at a higher concentration of 32K (Fig. 5, B and D) revealed that 30K could displace 32K already bound to actin in a competitive manner, despite the very poor actin-binding ability of 30K alone, suggesting that the interaction between 32K and 30K was strong enough to displace 32K bound to actin. The increased actin-binding of 30K in the presence of 32K was not due to heterodimerization of 32K/30K, because the two isoforms have previously been shown to form only homodimers under physiological conditions (*14*). Therefore, we propose that the increase was caused by the reinforced head-to-tail interaction between 32K and 30K. Specific viscosity measurements (Fig. 6) support this proposal: the specific viscosity of the mixture of 32K and 30K was higher than that of 32K alone. Recently, it has been shown that the interaction of the amino and carboxyl halves of different nonmuscle tropomyosin isoforms is important for tropomyosin functions, implying the significance of head-to-tail interactions of distinct isoforms of tropomyosin (*23*). Direct identification of the amino and carboxyl regions responsible for the head-to-tail interaction between 32K and 30K remains to be achieved.

Considering these results together, we suggest that the two isoforms are on the same actin filaments in an alternate fashion, based on the following evidence: (i) the actin-binding ratio of 32K to 30K reached 1:1 at saturation (Fig. 2). (ii) Below the saturation level the amount of actin-binding 30K did not exceed that of 32K (Fig. 5C). (iii) 32K and 30K each formed homodimers (*14*). This seems to be compatible with the previous report that both muscle-type and nonmuscle-type tropomyosin isoforms are co-localized at the cleavage furrow and peripheral region beneath the plasma membrane (*16*) and the molar ratio of 30K to 32K present in the egg tropomyosin fraction was about 1.5:1 (*14*). Therefore, it is likely that the actin-based structures consist of actin filaments to which 32K and 30K are bound alternately. We are trying to demonstrate the alternate arrangement of 32K and 30K by electron microscopy.

Recently, rat fibroblast TM-2 has been shown to increase the affinity of TM-1 for actin, although the molar ratio of TM-1 to TM-2 is 1:6 at saturated binding to actin (*39*). This result is consistent with ours, despite the difference of the binding mode. The fact that the increase in the actin-binding of one isoform by another can be recognized in tropomyosins from distantly related organisms may imply the functional importance of tropomyosin heterogeneity.

Other actin-binding proteins may affect the binding properties of tropomyosin isoforms to actin in a competitive or cooperative manner. Indeed, in sea urchin eggs many F-actin-binding proteins such as α-actinin (*9*), fodrin (*11*), fascin (*33, 34*), and 250-kDa protein (*12*) have been identified. It is interesting that 55-kDa protein, known as a fascin homologue in HeLa cells, has different effects on the actin-binding abilities of different species of tropomyosin isoforms (*34, 35*). Considering the involvement of those actin-binding proteins in the regulation of actin-based motile systems, it would be very interesting to know how 32K and 30K, which has only a weak ability to bind to actin by itself, interact with these actin-binding proteins. Therefore, it is important for the understanding of tropomyosin function in nonmuscle cells to detect and examine tropomyosin-binding proteins in sea urchin eggs, just as tropomodulin and caldesmon have been characterized in several nonmuscle cells (*36, 37*).

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