Troponin-Like Proteins from Muscles of the Scallop, *Aequipecten irradians*

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Ca^{2+} regulation of molluscan actomyosin adenosine triphosphatase is known to be associated with the myosin molecule. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, however, also suggests the possible presence of troponin, a thin-filament-linked Ca^{2+} -regulatory complex. In the present study, scallop troponin and tropomyosin were prepared and complexed with rabbit actin; the resulting synthetic thin filaments form a Ca^{2+} -dependent actomyosin adenosine triphosphatase with Ca^{2+} -insensitive rabbit myosin, indicating that the troponin in scallops is potentially functional. Scallop troponin I was isolated and mixed with chicken troponin C and troponin T, forming a functional hybrid troponin complex, indicating that scallop and vertebrate troponins may act by a common mechanism. Densitometry of sodium dodecyl sulphate/polyacrylamide gels reveals that in synthetic thin filaments there are larger amounts of troponin than are present in native thin filaments. Amounts present in the intact muscle were not determined.

Muscular contraction in all animals involves the interaction of actin, myosin and ATP; however, two very different systems, each responding to changes in Ca^{2+} concentration, exist to control this interaction. Regulatory proteins, which bind Ca^{2+} and confer a Ca^{2+} -dependence on the interaction of actomyosin with ATP, are located and act on the actin-containing thin filaments, the myosin or both (Lehman & Szent-Györgyi, 1975). Muscles in most animals studied so far are dual-regulated; the advantage of this type of Ca^{2+} regulation is presumably the large enhancement of the Ca^{2+} -dependence (Lehman *et al.*, 1974; Lehman & Szent-Györgyi, 1975). This results from both the myosin and the thin filaments, which necessarily must interact for ATPase activity and consequently for contraction to occur, being switched-off in the absence of Ca^{2+} . Thus animals with singly regulated muscles appear to be exceptions, and the reason for this is not clear.

The most well-characterized groups whose muscles are thought to be singly regulated are the vertebrates and the molluscs. Vertebrate striated muscle is considered to be solely thin-filament-regulated; however, many studies suggest the possibility of a simultaneously operating myosin control, although direct evidence is lacking (Werber *et al.*, 1972; Marimoto & Harrington, 1974; Hazelgrove, 1975; Lehman, 1977). Conversely, molluscs were thought to possess only the myosin-linked-system, showing no evidence of regulation by thin filaments (Kendrick-Jones *et al.*, 1970; Lehman *et al.*, 1972). More recent studies, however, demonstrated that small amounts of the thin-filament-linked control

protein, troponin, may occur in molluscs (Lehman & Szent-Györgyi, 1975). In view of our contention that dual regulation is advantageous, we decided to re-examine whether a functional thin-filament type of regulatory system may co-exist with the myosin control in molluscs.

Materials and Methods

Preparations

All preparations were carried out between 0 and 4°C.

Vertebrate proteins. Rabbit actin free of tropomyosin contamination was prepared by the method of Straub (1942) by using the modification of Drabikowski & Gergely (1964). Chicken breast tropomyosin was extracted and purified by the method of Bailey (1948) as modified by Lehman & Szent-Györgyi (1972a). Ca^{2+} -insensitive rabbit myosin was prepared from longissimus dorsi by the method of Szent-Györgyi (1951) and Mommaerts & Parrish (1951). Chicken breast troponin was prepared as described by Regenstein & Szent-Györgyi (1975). A fraction containing chicken breast troponin C and troponin T (troponin C-T) was prepared essentially by the method of Greaser & Gergely (1971). Troponin I was first eluted in the void volume from DEAE-Sephadex A-50 equilibrated with 50 mM-Tris/HCl buffer/2 mM-EDTA/6 M-urea, pH 8.0, and the troponin C and troponin T were removed together by application of 0.6 M-NaCl in the same buffer. The troponin C-T was then dialysed against 40 mM-NaCl/1 mM- MgCl_2 /0.1 mM- NaN_3 /5 mM-sodium phosphate buffer, pH 7.0. The troponin C-T was precipitated and was collected

Abbreviations used: ATPase, adenosine triphosphatase; Mg-ATPase, Mg^{2+} -activated ATPase; SDS, sodium dodecyl sulphate.

by centrifugation at 5000g for 2–3 min and resuspended in 0.6M-NaCl/5mm-phosphate buffer, pH7.0.

Scallop proteins. The striated adductor muscles of live bay scallops, *Aequipecten irradians*, were dissected free of smooth muscle and were used in all preparations described below.

(a) Scallop thin filaments. Native thin filaments were extracted from myofibrils by first homogenizing eight to ten muscles (approx. 25g wet wt.) with a Sorvall OmniMixer in 200ml of 100mM-NaCl/5mM-MgCl₂/1mM-EGTA/1mM-NaN₃/10mM-sodium phosphate buffer, pH7.0, and then centrifuging the homogenate at 15000g for 5 min; the myofibrillar pellets were redispersed in the above solution, and the centrifugation and redispersion steps were repeated three times more. Finally the pellets were homogenized in the above solution containing 5mM-ATP (pH6.0) and the pH of the homogenate was adjusted to pH6.0 with 1M-HCl. Particulate material and thick filaments were removed by sedimentation for 10 min at 30000g, followed by 120000g for 30 min. Thin filaments were then collected from the supernatant by centrifugation at 100000g for 3 h, and the pellets resuspended in 40mM-NaCl/5mM-MgCl₂/1mM-NaN₃/5mM-sodium phosphate buffer, pH7.5.

(b) 'Crude' scallop troponin-tropomyosin. For this, eight to twelve muscles were homogenized in 400ml of 0.3M-sucrose/0.1mM-NaCl/5mM-MgCl₂/1mM-EGTA/1mM-NaN₃/5mM-ATP/10mM-sodium phosphate buffer, pH6.0. The pH of the homogenate was readjusted to pH6.0 with 1M-HCl. Particulate material was removed by sedimentation for 10 min at 30000g, followed by 120000g for 30 min. The supernatant was then adjusted to 1mM-NaCl/10mM-sodium citrate/0.03% 2-mercaptoethanol and the pH lowered to 4.5 with 1M-HCl; precipitated material was removed by sedimentation at 30000g for 10 min. The pH of the supernatant was adjusted to 1.0 with 1M-HCl and after 30 min readjusted to pH7.3 with 1M-NaOH. [The pH1.0 acidification step denatures the contaminating actin and was used as an extra purification step, since troponin and tropomyosin survive this treatment (Hartshorne & Mueller, 1968).] After 30 min at pH7.3 the remaining precipitated protein was removed by sedimentation at 30000g for 10 min. Then 20g of (NH₄)₂SO₄ was added to each 100ml of supernatant and precipitated protein was removed by centrifugation for 10 min at 15000g. Crude troponin-tropomyosin was precipitated by an additional 30g of (NH₄)₂SO₄/100ml, the precipitate collected by centrifugation for 10 min at 15000g, and the pellet redissolved in and dialysed against 40mM-NaCl/5mM-MgCl₂/0.1mM-NaN₃/5mM-Tris/HCl buffer, pH7.7. Finally the preparation was clarified by a 1 h centrifugation at 200000g and the

pellet discarded. The amount of crude troponin-tropomyosin isolated varied from 60 to 100mg of protein.

(c) Synthetic thin filaments. Pure rabbit actin and crude scallop troponin-tropomyosin were mixed in a 1:10 ratio (w/w) in the above solution, additionally containing 0.7mM-ATP (pH7.7), and sedimented for 2.5 h at 120000g. The pellets were resuspended in the same solution used to resuspend the native thin filaments.

(d) Scallop troponin I. Solutions of crude troponin-tropomyosin were mixed with sulphopropyl-Sephadex C-50 in a ratio of 1mg of protein/1.5mg of dry Sephadex, and the mixture was dialysed against 90mM-NaCl/6mM-urea/0.03% 2-mercaptoethanol/50mM-Tris/HCl buffer, pH8.0. The Sephadex having bound the troponin I was sedimented, resuspended in the above solution containing 0.6M-NaCl, and removed by sedimentation at 5000g for 2–3 min. The supernatant containing pure troponin I was dialysed against 40mM-NaCl/1mM-MgCl₂/0.1mM-NaN₃/5mM-sodium phosphate buffer, pH7.5. The yield was between 0.2 and 1mg of troponin I per 100mg of crude troponin-tropomyosin.

(e) Hybrid troponin. Scallop troponin I was mixed with chicken troponin C-T in stoichiometric ratios

$$\left(\frac{23\,000 \text{ daltons}}{18\,000 + 41\,000 \text{ daltons}} \right)$$

and dialysed against 6M-urea/0.03% 2-mercaptoethanol/50mM-Tris/HCl buffer, pH8.0, and then against 40mM-NaCl/1mM-MgCl₂/0.1mM-NaN₃/5mM-sodium phosphate buffer, pH7.5. Molecular weights of scallop muscle proteins were determined by the method of Weber & Osborn (1969); chicken muscle proteins were determined previously by the same method (Lehman & Szent-Györgyi, 1972b).

Analysis

The actomyosin Mg-ATPase was measured in a pH-stat in 30mM-NaCl, pH7.5, at 25°C as described previously (Szent-Györgyi *et al.*, 1971). The duration of each assay was 4–5 min; 0.7mM-MgATP was used as substrate and total [MgCl₂] varied in different assays as indicated in the Results section. Ca²⁺-sensitivity (%) was measured by comparing the ATPase rates in the presence of 0.1mM-EGTA alone (ATPase_{EGTA}) and with 0.1mM-EGTA containing 0.2mM-CaCl₂ (ATPase_{Ca}) by using the following relationship:

$$\text{Ca}^{2+}\text{-sensitivity} = \frac{\text{ATPase}_{\text{Ca}} - \text{ATPase}_{\text{EGTA}}}{\text{ATPase}_{\text{Ca}}} \times 100$$

The ATPase activities of 1mg samples of rabbit myosin, mixed together with various amounts of rabbit actin or thin filaments, were assayed.

Ca²⁺-binding was determined as previously described by using a double-labelling technique (Kendrick-Jones *et al.*, 1970). For this [³H]glucose was used to measure the void volume of a sedimented pellet, and ⁴⁵Ca the bound calcium. The radioactivities of the isotopes used and the scintillation-counter settings were the same as previously described. Synthetic thin filaments were sedimented in 30 ml of 50 mM-NaCl/3 mM-MgCl₂/0.4 mM-⁴⁵Ca-EGTA / 0.0076 mM-EGTA / 10 mM-[³H]glucose/5 mM-imidazole buffer, pH 7.0, for 2 h at 100000g (isotopes supplied by New England Nuclear Corp., Boston, MA, U.S.A.). The free [Ca²⁺] in this solution should be 10 μM (Chaberek & Martell, 1959). The pelleted thin filaments were resuspended in water; 0.5 ml samples were dissolved in 6 ml of Aquasol (New England Nuclear Corp.) and their radioactivities counted in a Packard Tri-Carb scintillation counter.

Protein concentrations were measured by the method of Lowry *et al.* (1951), with bovine serum albumin standards, which in turn were standardized by nitrogen determinations (Strauch, 1965). SDS/polyacrylamide-gel electrophoresis was performed by the method of Weber & Osborn (1969) with Coomassie Brilliant Blue as a routine stain. Densitometry (at 640 nm) of 10% polyacrylamide gels stained with Fast Green FCF (BDH Chemicals, Poole, Dorset, U.K.) was performed on a Gilford 240 spectrophotometer with the use of a linear transport adaptor and a Beckman DU monochromator and recorder.

Results

Crude troponin-tropomyosin

Although SDS/polyacrylamide-gel electrophoresis of scallop muscles show bands that could represent troponin subunits (Lehman & Szent-Györgyi, 1975), attempts to isolate troponin by standard procedures

(Hartshorne & Mueller, 1969; Regenstein & Szent-Györgyi, 1975) have not been successful. In the present study, we have prepared a soluble fraction of scallop muscle proteins that, when added in large amounts (8–10 mg/3 mg of rabbit myosin/1 mg of rabbit actin), renders the rabbit actomyosin ATPase Ca²⁺-dependent. SDS/polyacrylamide-gel electrophoresis of the preparation shows a large number of bands including actin, tropomyosin and three other bands also found on gels of native thin filaments (compare Plate 1, gels *a* and *b*). These three bands are not present in crude troponin-tropomyosin preparations in equimolar proportions, accounting for the large amounts needed to observe Ca²⁺-sensitivity. Even though actin is present in this fraction, it is denatured, and the preparation has neither ATPase activity of its own nor activates the Mg-ATPase of rabbit myosin.

Synthetic scallop thin filaments

We wished to determine which of the proteins of this crude troponin-tropomyosin preparation interact with rabbit F-actin and whether this interaction is responsible for the Ca²⁺-sensitivity observed. SDS/polyacrylamide gels show that only four proteins complex and co-sediment with rabbit F-actin, namely tropomyosin and the three additional components found on native thin filaments (Plate 1, gels *c* and *d*). The two bands of lowest molecular weight migrate with similar mobility to vertebrate troponin I and troponin C. The third band migrates slightly slower than does actin. Densitometry of these synthetic thin filaments reveals larger amounts of the troponin I- and troponin C-like proteins, relative to tropomyosin and actin, than are present in rabbit or *Limulus* thin filaments (Table 1). This may reflect some non-specific interaction of these two components when complexing to form synthetic thin filaments. The synthetic thin filaments bind an average of 1.5 μmol of Ca/g of protein at 10 μM-Ca²⁺.

Table 1. *Densitometry of synthetic thin-filament gels*

Results are averages of stain intensities, relative to tropomyosin, of gel bands from four different thin-filament preparations. Ranges of values are given in parentheses. For each thin-filament preparation, a series of gels, containing from 5 to 40 μg of total protein, were scanned at 640 nm. The relative ratios of stain intensities for the components of a particular gel were computed by determining peak areas, and the respective ratios were then averaged for the series. Excluded from the computation were peaks with maximum intensity lower than 0.02 and higher than 0.32 A unit. For the purposes of comparison, comparable data from rabbit myofibrils (Potter, 1974) and *Limulus* native thin filaments (Lehman *et al.*, 1976) are also included.

	Relative stain intensity			
	Actin	Tropomyosin	Troponin I	Troponin C
Synthetic scallop thin filaments	213 (184–266)	70	44 (37–57)	31 (28–35)
Rabbit myofibrils	207	70	25	10
<i>Limulus</i> native thin filaments	228	70	39	11

Table 2. *ATPase activities of synthetic scallop thin filaments and rabbit myosin*

Complete sets of ATPase assays were performed on two different thin-filament preparations; the Table shows the results obtained after varying $[MgCl_2]$ and the ratio of thin filaments/myosin. Also shown are controls with vertebrate proteins. ATPase activity is expressed in μmol of ATP split/min per mg of rabbit myosin; values in parentheses are the Ca^{2+} -sensitivities (%) of each preparation. Two additional preparations of synthetic thin filaments, which were used for Ca^{2+} -binding experiments, gave Ca^{2+} -sensitivities of 75 and 82% at the higher $[MgCl_2]$.

Sample assayed	Mg-ATPase (assayed in 30mM-NaCl/1 mM-MgCl ₂ / 0.7 mM-ATP)			Mg-ATPase (assayed in 30mM-NaCl/3 mM-MgCl ₂ / 0.7 mM-ATP)		
	+EGTA	+Ca ²⁺	Ca ²⁺ -sensitivity	+EGTA	+Ca ²⁺	Ca ²⁺ -sensitivity
(a) 0.5mg of synthetic thin filaments+ 1.0mg of rabbit myosin	0.43	0.50	14 (5, 24)	0.11	0.35	69 (63, 76)
(b) 1.0mg of synthetic thin filaments+ 1.0mg of rabbit myosin	0.70	0.85	16 (6, 26)	0.11	0.38	71 (65, 76)
(c) 0.33 mg of rabbit actin+0.16 mg of chicken tropomyosin+0.26 mg chicken troponin+1.0mg of rabbit myosin	0.24	0.90	73	0.09	0.63	86
(d) 0.33 mg of rabbit actin+0.16 mg of chicken tropomyosin+1.0mg of rabbit myosin	0.53	0.53	0	0.46	0.47	2

Table 3. *Effect of scallop troponin I on rabbit actomyosin ATPase*

Results are averages of three preparations of scallop troponin I and two preparations of chicken troponin C-T. Hybrid troponin signifies troponin components that were mixed together and dialysed against 6M-urea and then against low ionic strength as indicated in the Materials and Methods section. Other mixtures of troponin components indicated in this Table were carried out at 0.6M-NaCl. ATPase activity is expressed in μmol of ATP split/min per mg of myosin; values in parentheses are the individual Ca^{2+} -sensitivities.

Sample assayed	Mg-ATPase (assayed in 30mM-NaCl/ 3 mM-MgCl ₂ /0.7 mM-ATP)		
	+EGTA	+Ca ²⁺	Ca ²⁺ -sensitivity
(a) 0.33 mg of rabbit actin+0.16 mg of chicken tropomyosin+0.43 mg of chicken troponin C-T+1.0mg of rabbit myosin	0.48	0.43	—
(b) 0.33 mg of rabbit actin+0.16 mg of chicken tropomyosin+0.16 mg of scallop troponin I+1.0mg of rabbit myosin	0.082	0.085	—
(c) 0.33 mg of rabbit actin+0.16 mg of chicken tropomyosin+0.43 mg of chicken troponin C-T+0.16 mg of scallop troponin I+1.0 mg of rabbit myosin	0.35	0.45	22
(d) 0.33 mg of rabbit actin+0.16 mg of chicken tropomyosin+0.32 mg of hybrid troponin+ 1.0mg of rabbit myosin	0.13	0.44	70 (68, 73)

ATPase assays show that the synthetic thin filaments activate and confer a Ca^{2+} -dependence on the Mg-ATPase of rabbit myosin, but only when the actomyosin assay solution contains 3mM-MgCl₂. This is 3 times the MgCl₂ concentration previously used (Szent-Györgyi *et al.*, 1971), and 1mM-

MgCl₂ yields a very low Ca^{2+} -sensitivity [Table 2 (a)]. The increase in Ca^{2+} -sensitivity observed at higher Mg^{2+} concentration is not surprising, in view of a large increase in Ca^{2+} -sensitivity conferred by vertebrate troponin-tropomyosin at elevated Mg^{2+} concentration [Table 2 (c)]. As expected,

EXPLANATION OF PLATE I

SDS/polyacrylamide-gel electrophoresis of various preparations used in this study

Gels (10%) were stained with Coomassie Brilliant Blue. (a) Crude scallop troponin-tropomyosin (40 µg); (b) native scallop thin filaments (approx. 50 µg); (c) synthetic scallop thin filaments (48 µg); (d) synthetic scallop thin filaments (30 µg); (e) scallop troponin I (3 µg); (f) chicken troponin C-T (16 µg). Abbreviations: A, actin; TM, tropomyosin; X, a protein that may be the scallop troponin T; TN-I, scallop troponin I; TN-C, TN-T, chicken troponin C and troponin T.

Table 4. *ATPase activities of scallop thin filaments and rabbit myosin*

ATPase assays were performed on five different thin filament preparations: the Table shows the results obtained after varying $[MgCl_2]$ and the ratio of thin filaments/myosin. Also shown are controls with vertebrate proteins. Variation in the Ca^{2+} -sensitivity in the different preparations is indicated by values in the parentheses. ATPase activity is expressed as μmol of ATP split/min per mg of rabbit myosin.

Sample assayed	Mg-ATPase (assayed in 30mM-NaCl/ 1 mM- $MgCl_2$ /0.7 mM-ATP)			Mg-ATPase (assayed in 30mM-NaCl/ 3 mM- $MgCl_2$ /0.7 mM-ATP)		
	+EGTA	+ Ca^{2+}	Ca^{2+} -sensitivity	+EGTA	+ Ca^{2+}	Ca^{2+} -sensitivity
(a) 0.5 mg of scallop thin filaments + 1.0 mg of rabbit myosin	0.89	0.92	3 (0–6)	0.22	0.33	32 (12–39)
(b) 1.0 mg of scallop thin filaments + 1.0 mg of rabbit myosin	0.98	1.14	14 (5–21)	0.24	0.39	38 (26–51)
(c) 3.0 mg of scallop thin filaments + 1.0 mg of rabbit myosin	0.85	1.01	17 (4–27)	0.18	0.42	56 (52–60)
(d) 3.0 mg of rabbit actin + 1.0 mg rabbit myosin	—	—	—	0.71	0.74	4
(e) 3.0 mg of rabbit actin + 1.5 mg of chicken tropomyosin + 1.0 mg of rabbit myosin	—	—	—	0.63	0.64	2

ATPase assays of troponin-free actomyosin do not show a Ca^{2+} -dependence at either Mg^{2+} concentration [Table 2 (d)].

Scallop troponin I

We have not been able to fractionate the troponin from tropomyosin in the crude troponin-tropomyosin preparation, but we have succeeded in purifying the 23000-dalton component present on thin filaments (Plate 1, gel e). By analogy to the molecular weights of vertebrate troponin components, this protein may be troponin I. Indeed, addition of this component to pure rabbit actin and myosin leads to a troponin I-like Ca^{2+} -insensitive inhibition of the actomyosin ATPase [Table 3 (b)]. Addition of vertebrate troponin C–T to the mixture relieves the troponin I-imposed inhibition, but the ATPase is only marginally Ca^{2+} -sensitive [Table 3 (c)]. However, with prior mixture of the vertebrate troponin C–T and the scallop troponin I in 6M-urea, and then removal of the urea by dialysis, a complex forms that does confer a Ca^{2+} -dependence on the vertebrate actomyosin ATPase [Table 3 (d)]. We have not succeeded, as yet, in purifying the 18000-dalton component, which may represent scallop troponin C, nor the 48000-dalton component, possibly equivalent to troponin T.

Native scallop thin filaments

Since synthetic scallop thin filaments impart Ca^{2+} -sensitivity only at elevated Mg^{2+} concentration, we investigated whether native thin filaments act

similarly. Again, Ca^{2+} -sensitivity is only observed at higher Mg^{2+} concentration. However, native thin filaments confer a Ca^{2+} -dependence on rabbit myosin with consistency only when thin filaments are present in excess over rabbit myosin [3 mg of thin filaments/mg of myosin; compare Table 4 (a), (b) and (c)], whereas the Ca^{2+} -sensitivity obtained with synthetic thin filaments does not depend on the presence of excess amounts [compare with Table 2 (a) and (b)]. This result is not an artifact relating simply to the presence of the extra actin, since troponin-free actomyosin is not Ca^{2+} -sensitive even when additional actin is present [Table 4 (d) and (e)].

Discussion

It is generally recognized that a myosin-linked Ca^{2+} -regulatory system occurs in molluscs (Kendrick-Jones *et al.*, 1970). On the other hand, it is not firmly established whether in molluscs a thin filament-linked regulatory system co-exists with the myosin regulation, as occurs in most if not all other invertebrates (Lehman & Szent-Györgyi, 1975; Lehman, 1977). SDS/polyacrylamide gels of scallop thin filaments do show small amounts of three bands in addition to actin and tropomyosin, and resemble the band pattern of Ca^{2+} -regulated thin filaments (Lehman & Szent-Györgyi, 1975). However, the weight ratio of these bands to tropomyosin was considered insufficient to regulate actin, and therefore it was questionable whether these proteins represented functional troponin (Lehman & Szent-Györgyi, 1975). In the present study, we have adopted another approach and attempted to evaluate whether these proteins have troponin-like activity.

We have tested a fraction from scallops that behaves analogously to vertebrate troponin-tropomyosin. The proteins of this crude troponin-tropomyosin-like preparation are soluble by themselves, but are able to interact with pure actin; the resultant synthetic thin filaments then bind Ca^{2+} and render the Mg-ATPase of myosin Ca^{2+} -sensitive. The evidence therefore suggests that proteins exist in molluscs that are capable of regulating actin.

It seems likely that the three proteins that, in addition to tropomyosin, bind to thin filaments represent troponin subunits. This conclusion is supported by the results with the purified 23000-dalton component. This protein behaves in a fashion similar to vertebrate troponin I and forms a functional hybrid with vertebrate troponin C and troponin T, conferring a Ca^{2+} -dependence on tropomyosin-containing rabbit actomyosin. This interchangeability of subunits implies that troponin in scallops may operate by the same mechanism as vertebrate troponin.

The Ca^{2+} -sensitivity conferred by the synthetic thin filaments is observed only at Mg^{2+} concentrations greater than used in previous studies. Eaton *et al.* (1975) have found that vertebrate tropomyosin binds more tightly to actin at elevated Mg^{2+} concentrations, and similarly, in our study, the additional Mg^{2+} may be essential for the binding of either scallop troponin or tropomyosin to the thin filaments, or may be involved in establishing the proper configuration of troponin, allowing Ca^{2+} -sensitive interactions to occur. Alternatively, the Mg^{2+} may induce myosin to be responsive to the Ca^{2+} -sensitive reaction on the thin filament. It is noteworthy that, although higher Mg^{2+} concentration is not necessary for the Ca^{2+} -dependent response attributable to vertebrate troponin-tropomyosin, elevated Mg^{2+} concentration increases the Ca^{2+} -sensitivity of the vertebrate system as well.

Results from the present investigation, showing native scallop thin filaments conferring Ca^{2+} -sensitivity only when present in excess, are difficult to interpret. The result cannot be explained by straightforward arguments that insufficient troponin is present, since one then would expect extra thin filaments to increase the population of troponin-free actin and therefore to decrease Ca^{2+} -sensitivity, not increase it, as is observed. The significance of this effect remains to be determined.

Our experiments do not resolve whether sufficient troponin exists in scallops to regulate the thin filaments *in vivo*, but do demonstrate that the troponin present is functional. Szent-Györgyi (1976) has reported less troponin is detectable in the deep-sea scallop, *Placopecten magellanicus*, than is present in *Aequipecten*, and in a study of our own we have confirmed this finding. Since tropomyosin and troponin may dissociate or be degraded during

preparation of thin filaments, we think that a general re-examination of the stoichiometry of components on molluscan thin filaments is warranted.

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