Proc. Natl. Acad. Sci. USA
Vol. 75, No. 8, pp. 3588–3592, August 1978
Biochemistry

Specific phosphorylation at serine-283 of α tropomyosin from frog skeletal and rabbit skeletal and cardiac muscle

(coiled coil structure/polymerization/aggregation/troponin binding/calcium regulation)

A. MAK*, L. B. SMILLIE*, AND M. BÁRÁNY†

* Medical Research Council of Canada Group on Protein Structure and Function, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7; and † Department of Biological Chemistry, University of Illinois at the Medical Center, Chicago, Illinois 60612

Communicated by William F. Harrington, April 28, 1978

ABSTRACT **Tropomyosin, extracted from the leg muscle of frogs that had been injected with [^{32}P]orthophosphate, was fractionated into two components, α and β, on a CM-cellulose column. Radioactivity was associated only with the α component. A single phosphorylation site was located at serine-283 (penultimate at the COOH-terminal end) of the frog α tropomyosin. The same phosphorylated peptide was recovered in low yields from both rabbit skeletal α and cardiac tropomyosin. The presence of covalently bound phosphate in α tropomyosin and its absence in the β component of rabbit skeletal muscle was suggested by ^{31}P NMR spectroscopy. The amino acid sequences around the phosphorylation sites of frog and rabbit tropomyosin are identical. Because this sequence is not similar to any other known phosphorylation site in proteins, this indicates the existence of either specific kinase or phosphatase that can distinguish between α and β tropomyosins. In a model proposed for the head-to-tail overlap of α tropomyosin molecules, one *O*-phosphoserine-283 residue could form a salt linkage with lysine-6 on one side of the overlap region and another with lysine-12 on the other side. This would predict a difference in the stability of polymers of phosphorylated and nonphosphorylated αα and αβ dimers of tropomyosin.**

Skeletal and cardiac muscle tropomyosin, a two-stranded, coiled-coil protein of molecular weight 66,000, is known to be intimately involved in the calcium regulatory system of striated muscle. Situated in the two grooves of the double-stranded structure of F-actin, it forms a long filament by aggregation of individual molecules involving an overlap of eight or nine amino acid residues at the NH_2- and COOH-terminal ends. Throughout its length each tropomyosin molecule interacts with seven actin monomers on each of the two strands of F-actin. Each tropomyosin molecule also binds one mole of troponin complex probably at a position about one-third of the distance from its COOH-terminal end. The binding of calcium to the Tn-C component of troponin is believed, by a series of conformational changes, to lead to a movement of the average position of tropomyosin toward the center of the groove of the thin filament and away from the center of mass of the actin. This movement of tropomyosin has been postulated to expose the myosin-binding region on actin and relieve the steric blocking by tropomyosin in the inhibited state, permitting the contractile events to occur (for a review with appropriate original references, see ref. 1).

Tropomyosin from rabbit skeletal muscle is composed of two major forms, α and β tropomyosin (2), present in a molar ratio of about 3.5:1. Isoelectric focusing (2–6) and sequence evidence has shown that additional forms are present but these are probably minor variants of the two major components α and β, perhaps involving only one amino acid substitution and certainly not more than a few. The physiological significance of these two major forms of tropomyosin is obscure. In a comparison of several muscle types, Cummins and Perry (7) have shown that the ratio of the α and β components is characteristic of the muscle source. Skeletal muscles consist predominantly of white fibers that contain tropomyosins in which α:β is appreciably greater than 1; in the slower red muscles the relative amounts of the α component are lower, the ratios falling to values close to 1. In cardiac muscle the relative amounts of α and β have been reported to be dependent on the size and contraction speed of the heart (8). In large and slowly beating hearts (pig, sheep, and human), α:β varies between 4.2:1 and 4.8:1; in small and faster beating hearts (rabbit, guinea pig, rat, and dog), the β component appears to be absent. We have recently shown from sequence analyses (W. G. Lewis and L. B. Smillie, unpublished results) that rabbit cardiac tropomyosin is at least 90% α component and identical to the rabbit skeletal protein.

In recent years, evidence has accumulated to show that some myofibrillar proteins undergo phosphorylation and dephosphorylation. Thus, phosphorylation of the troponin components Tn-T and Tn-I as well as of the myosin 18,000 dalton light chain *in vivo* and *in vitro* has been well documented (for recent review, see ref. 9). Recently one of our laboratories has demonstrated that tropomyosin, isolated from frogs previously injected with [^{32}P]orthophosphate, is significantly phosphorylated at a serine residue (10). We show that this phosphate incorporation occurs at serine-283, the penultimate residue in the sequence of frog α tropomyosin, but that no radioactivity is present in the β component. This is true even though the sequences about serine-283 of the α and β components are similar. We have also demonstrated a low level of phosphorylation at the same site in the α tropomyosin of rabbit skeletal and cardiac muscle but have been unable to show this in β tropomyosin, either by peptide mapping or by ^{31}P NMR spectroscopy.

MATERIALS AND METHODS

Tropomyosin was isolated from the leg muscles of frogs which had been injected with [^{32}P]orthophosphate as described by Ribolow and Bárány (10); iodoacetamide (1 mM) was added throughout the isolation of the ethanol/ether dried powder as well as throughout the purification procedures of tropomyosin. Tropomyosin was prepared from rabbit skeletal and cardiac muscle as described (11). Nucleic acid contamination was removed by chromatography on a DEAE-cellulose column as described by Woods (12). Carboxymethylation of the frog and rabbit tropomyosin was carried out as before (11).

The carboxymethylated tropomyosins from frog and rabbit skeletal muscle were fractionated into their α and β components by a modified method of Cummins and Perry (2). Carboxymethylated tropomyosin (200 mg) was dissolved in 15 ml of 0.05

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

M sodium formate, pH 4.0/1.0 mM dithiothreitol/8.0 M urea and dialyzed against the same buffer overnight before application to a CM-cellulose column (Whatman CM32), 2.0 × 22 cm, which had been equilibrated with at least two column volumes of the starting buffer. The protein was eluted by a linear gradient of 0.0–0.2 M NaCl in 1500 ml of buffer. The eluant was monitored at 280 nm. For measuring radioactivity, 250 μl from alternate tubes was mixed with 100 μl of water and 10 ml of Biofluor (New England Nuclear) and counted on a Beckman scintillation counter for 20 min. The purity of the separated α and β components was established on sodium dodecyl sulfate/urea/polyacrylamide gels as described by Sender (13).

Tryptic peptide maps were obtained by two-dimensional high-voltage paper electrophoresis. The first and second dimensions were run at pH 6.5 and 1.8, respectively (14). The map was stained with cadmium/ninhydrin reagent (15).

The ^{32}P-labeled tryptic peptide from frog tropomyosin was located by radioautography with Kodak x-ray film. The presence of *O*-phosphoserine in peptides was determined by partial acid hydrolysis of the phosphorylated peptides in 6 N HCl at 110° for 2 hr. The resulting hydrolysate was fractionated by high voltage paper electrophoresis at pH 1.8. Separation of *O*-phosphoserine and *O*-phosphothreonine was achieved in this system.

Covalently bound phosphate content was determined as described by Stull and Buss (16). Protein determination was performed by amino acid analysis, based on 33 and 36 alanine residues per chain of α and β tropomyosin, respectively. The NH_2-terminal residues were determined by dansylation (17) and amino acid analysis was performed on a Durrum D500 analyzer as described (18).

Tryptic (trypsin-TPCK, Worthington Biochemical Co.) and *Staphylococcus aureus* protease (a generous gift from G. Drapeau, University of Montreal) digests were carried out in 0.2 M NH_4HCO_3, pH 8.1 at 37° for 4–18 hr. The enzyme to substrate ratio was 1:100. The resulting peptides were purified by high-voltage paper electrophoresis at pH 6.5, 1.8 or 3.5. Digestion with *Escherichia coli* alkaline phosphatase (kindly supplied by B. Sykes) was carried out in 0.05 M Tris·HCl, pH 8.0/10 mM $MgCl_2$/0.15 M NaCl for 2 hr at 37°. The enzyme to substrate ratio was 1:100.

The ^{31}P NMR spectra were taken at 109.3 MHz on a Bruker HX5-270 NMR spectrometer operating in the Fourier mode. The instrument settings were: acquisition time, 0.41 s; delay between acquisitions, 1.6 s; sweep width, 5000 Hz; spectrum size, 4096 data points; line broadening, 10 Hz. The samples contained α or β tropomyosin (about 40 mg/ml) in 10 mM piperazine-*N*,*N*′-bis-(2-ethanesulfonic acid)/10 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid/0.5 mM sodium 2,2-dimethyl-2-silapentane-5-sulfonate/1 mM EDTA, at pH 7.0.

RESULTS AND DISCUSSION

Frog skeletal tropomyosin exhibited a double-band pattern on sodium dodecyl sulfate/urea/gel electrophoresis at pH 7.0. Consistent with the nomenclature of the tropomyosin components in rabbit skeletal muscle (2), the faster moving component is designated α and the slower β.

The elution profile of the fractionation of the ^{32}P-labeled frog skeletal tropomyosin on a CM-cellulose column (Fig. 1) showed that β and α tropomyosins were eluted at 0.055 M and 0.130 M NaCl, respectively. Both fractions were homogeneous as judged by sodium dodecyl sulfate/urea/gel electrophoresis. On the basis of the total absorbance under each peak, the ratio of α to

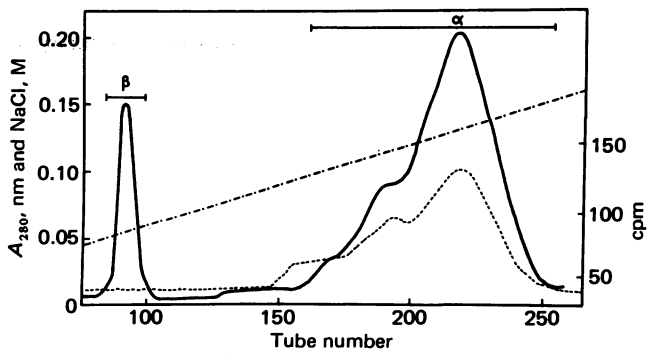

FIG. 1. Fractionation of ^{32}P-labeled frog skeletal tropomyosin on a CM-cellulose column (2.0 × 22 cm) equilibrated with 0.05 M sodium formate, pH 4.0/8 M urea/1.0 mM dithiothreitol. The protein was eluted by a 0.0–0.2 M linear gradient of NaCl (----) in 1500 ml of buffer. Fractions (4.5 ml/tube) were collected and monitored by measuring the absorbance at 280 nm (—) and radioactivity in cpm (- - - -). Fractions were pooled as indicated.

β was 6.2:1. Interestingly, radioactivity was associated only with the α component.

Although the α component was found only in the second peak (fractions 145–245 of Fig. 1) as judged by sodium dodecyl sulfate/urea/gel electrophoresis, this fraction appeared to be heterogeneous based on the asymmetry of both the ^{32}P and absorbance profiles. This apparent heterogeneity is probably a reflection of the partial separation of phosphorylated α tropomyosin from nonphosphorylated α component in this system and the formation of disulfide linked dimers of phosphorylated and nonphosphorylated forms in this particular preparation of carboxymethylated frog tropomyosin. A small proportion of noncarboxymethylated material in our preparation would permit the formation of such dimers which are known to be eluted separately from the monomeric α tropomyosin in this system (ref 19; A. Mak and L. B. Smillie, unpublished results). However the possiblity that there are additional minor variants of α tropomyosin in frog muscle, as is the case in rabbit skeletal muscle, cannot be excluded at this time.

Radioautographs of the tryptic maps of the α and β frog tropomyosin revealed one single radioactive spot on the map of the α component but none on the β map (Fig. 2). The ^{32}P-labeled peptide from frog α tropomyosin, αT1, was isolated and purified by high-voltage paper electrophoresis. The properties of this peptide are shown in Table 1. The NH_2-terminal residue

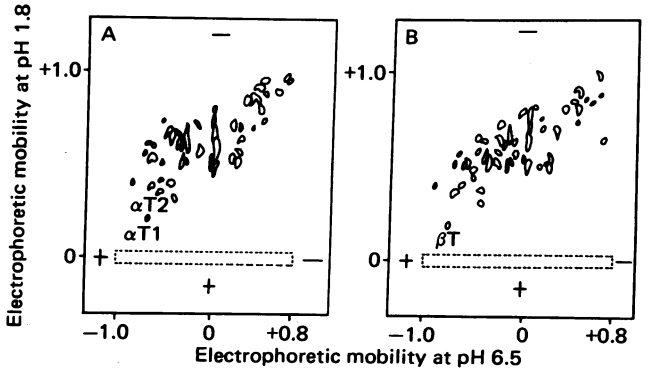

FIG. 2. Tryptic peptide maps of α (*A*) and β (*B*) frog skeletal tropomyosin by high-voltage paper electrophoresis. The first dimension was performed at pH 6.5 and the second dimension, at pH 1.8. Electrophoretic mobility at pH 6.5 was measured with respect to Asp which was assigned a value of −1.0; at pH 1.8, Lys was assigned a value of +1.0. The radioactive peptide, αT1, is indicated by a solid spot.

Table 1. Amino acid compositions* and other properties of phosphorylated and nonphosphorylated peptides derived from COOH-terminal ends of frog and rabbit tropomyosins

Amino acid	Frog α tropomyosin				Frog β tropomyosin	Rabbit cardiac tropomyosin		
	αT1	αT2	αT1-Sa	αT1-Sb	βT	CT1	CT1-Sa	CT1-Sb
Asp	2.8(3)	2.9(3)	—	2.9(3)	4.0(4)	3.1(3)	—	3.2(3)
Thr	0.9(1)	0.8(1)	—	1.0(1)	1.0(1)	0.9(1)	—	1.2(1)
Ser	1.5(2)	1.6(2)	1.0(1)	0.8(1)	1.8(2)	1.5(2)	1.0(1)	1.0(1)
Glu	2.7(2)	2.2(2)	1.8(2)	—	2.2(2)	2.3(2)	2.0(2)	—
Ala	2.0(2)	2.0(2)	1.0(1)	1.0(1)	2.2(2)	2.0(2)	1.0(1)	1.0(1)
Ile	1.8(2)	1.8(2)	1.0(1)	0.9(1)	1.8(2)	1.7(2)	1.0(1)	1.1(1)
Leu	2.5(2)	2.2(2)	—	2.0(2)	2.9(3)	1.9(2)	—	1.7(2)
His	0.7(1)	0.8(1)	—	0.9(1)	—	0.7(1)	—	1.0(1)
Met	0.8(1)	0.7(1)	—	0.9(1)	—	0.8(1)	—	1.1(1)
Total	(16)	(16)	(5)	(11)	(16)	(16)	(5)	(11)
Yield %	3.6	7.4			12.2	1.4		
Mobility†	−0.68	−0.55	−0.71	−0.53	−0.65	−0.68	−0.71	−0.53
Calculated charge‡	>−4	>−3	−2	−3	−4	>−4	−2	−3
NH_2 terminus	Ala	Ala	Ala	Leu	Ala	Ala	Ala	Leu
O-phosphoserine	Yes	No	No	Yes	No	Yes	No	Yes

* Residues per mole of peptide. Integral values in parentheses are those expected from known sequences of rabbit skeletal α and β and cardiac tropomyosins.
† Electrophoretic mobilities at pH 6.5 relative to Asp = −1.0.
‡ Charge calculated according to Offord (20).

and amino acid composition of this peptide are essentially identical to those of the COOH-terminal tryptic peptide, residues 269–284, from rabbit skeletal α tropomyosin and cardiac tropomyosin (W. G. Lewis and L. B. Smillie, unpublished data) (Fig. 3). Partial acid hydrolysis of this peptide yielded *O*-phosphoserine. In addition, a nonradioactive peptide, αT2, with the same NH_2 terminus and amino acid composition (Fig. 1 and Table 1) as αT1 was isolated. Devoid of *O*-phosphoserine, it represents the nonphosphorylated form of the COOH-terminal tryptic peptide of α tropomyosin (residues 269–284). The yields of αT1 and αT2 were 3.6% and 7.4%, respectively, indicating that about 30% of the frog α tropomyosin was in the phosphorylated form. To establish which of the two serines (residue 271 or 283) was phosphorylated, αT1 was digested with *S. aureus* protease in 0.2 M NH_4HCO_3, pH 8.1. Under these conditions cleavage was limited to glutamate residues (21). Two major peptides, αT1-Sa and αT1-Sb, were isolated and purified by high voltage paper electrophoresis at pH 6.5 and 1.8. Their properties, amino acid compositions, and NH_2 termini are given in Table 1. Peptide αT1-Sa clearly represents residues 269–273 and αT1-Sb corresponds to residues 274–284 of the rabbit skeletal α tropomyosin sequence. Radioactivity and *O*-phosphoserine were found only in αT1-Sb. These results clearly demonstrate that serine-283, penultimate to the COOH terminus of the molecule, is uniquely and specifically phosphorylated in frog α tropomyosin *in vivo* and that the COOH-terminal sequence of this component is identical to that of rabbit skeletal and cardiac α tropomyosin.

The finding that frog β tropomyosin is devoid of radioactivity might indicate that its COOH-terminal sequence is markedly different from that of α tropomyosin or that serine-283 is replaced by another amino acid. To check this possibility a search was undertaken for the COOH-terminal tryptic peptide of the β component. Assuming a similar molecular size and electrophoretic mobility as the corresponding peptide in rabbit β tropomyosin, it was predicted that it would correspond to peptide βT of Fig. 2*B*. After isolation and purification by high voltage electrophoresis it was shown to have the same properties, NH_2 terminus and amino acid composition (Table 1) as the same peptide from rabbit β tropomyosin (Fig. 3). It may be concluded that the COOH-terminal sequences of frog α and β tropomyosins are identical to those of the same components from rabbit. This may reflect a conservation of the head-to-tail polymerization characteristics of tropomyosin in the two species.

The finding that frog α tropomyosin was phosphorylated *in vivo* raised the question of whether rabbit skeletal and cardiac tropomyosins also undergo this chemical modification. In our previous amino acid sequence analysis of the rabbit tropomyosins from both skeletal and cardiac tissues (refs. 3–6; unpublished data) we had found no evidence that serine-283 occurred in a phosphorylated form. However, if, in the isolation of these tropomyosins, the action of phosphatase(s) had led to extensive dephosphorylation, such phosphorylated peptide(s) would have been present in low yields and would have been overlooked in the isolation and purification of the major peptides employed for the establishment of the amino acid sequence. To reinvestigate this possibility, rabbit skeletal α and β and cardiac tropomyosins, prepared in the absence and presence of phosphatase inhibitors, were analyzed for covalently linked phosphate

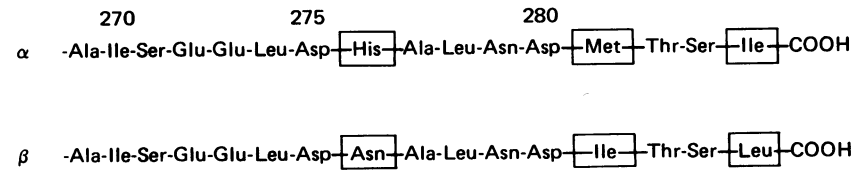

FIG. 3. Comparison of the amino acid sequences of the COOH-terminal tryptic peptides from frog and rabbit skeletal α and β tropomyosins. Replacements are enclosed in boxes.

by examining their tryptic peptide maps. When prepared in the absence of phosphatase inhibitors, skeletal α, β, and cardiac tropomyosins were found to contain 0.08 ± 0.01, 0.05 ± 0.01, and 0.09 ± 0.01 moles of covalently bound phosphate per 33,000 daltons, respectively (averages of three analyses). When phosphatase inhibitors were included in the extraction procedure, no significant increase in phosphate content was detected in the skeletal α and β components (0.11 ± 0.01 and 0.04 ± 0.01 mol per 33,000 daltons, respectively), whereas 0.21 ± 0.01 mol of phosphate was found in the rabbit cardiac tropomyosin. These values may be compared with the up to 0.5 mole of phosphate found per 33,000 daltons of frog tropomyosin when prepared in the presence of 1 mM iodoacetamide. To further assess the question of whether these low levels of phosphate content were attributable to the presence of a phosphorylated serine-283 residue in these proteins, tryptic peptide maps of the skeletal α, β, and cardiac tropomyosins were prepared. The maps of the skeletal α and cardiac proteins were similar to the frog α component and showed a ninhydrin positive spot with the same mobility as the phosphorylated COOH-terminal peptide (αT1) of frog α tropomyosin. After digestion of the cardiac tropomyosin with *E. coli* alkaline phosphatase, this spot disappeared from the tryptic map. Amino acid analyses and NH_2-terminal and *O*-phosphoserine determinations (Table 1) indicated that this peptide, CT1, from cardiac tropomyosin was identical to αT1 from frog α tropomyosin. *S. aureus* protease digestion of this peptide yielded two peptides, CT1-Sa and CT1-Sb, that had the identical NH_2-terminal residues and amino acid compositions as αT1-Sa and αT1-Sb, respectively, from frog α tropomyosin. The presence of *O*-phosphoserine in only CT1-Sb showed that serine-283 in rabbit cardiac tropomyosin was the site of phosphorylation. Analysis of the same peptide from the tryptic map of rabbit skeletal α component also demonstrated the presence of *O*-phosphoserine. However, we were unable to detect the presence of a peptide corresponding to a phosphorylated form of the COOH-terminal peptide of the β form of skeletal tropomyosin.

Further evidence for the phosphorylation of rabbit skeletal α tropomyosin was provided by ^{31}P NMR studies. A resonance was observed at the same frequency as that for a standard solution of *O*-phosphoserine, about 1.7 ppm downfield from the inorganic phosphate signal. After digestion of the protein with *E. coli* alkaline phosphatase, the ^{31}P signal was shifted upfield to the frequency of inorganic phosphate. No corresponding signal could be detected with a solution of β tropomyosin. These studies demonstrate clearly that both rabbit skeletal α and cardiac tropomyosin exist as partially phosphorylated species and suggest, but not conclusively, that the skeletal β form does not. The low value of covalently bound phosphate detected in β tropomyosin may arise from low levels of nucleic acid or other contamination in the preparation that we have been unable to eliminate.

The finding that α tropomyosin isolated from frog and rabbit skeletal muscle is phosphorylated, whereas the β component at least in frogs is not, is surprising, because the amino acid sequences of these two components are similar. In the immediate region of serine-283, present in both α and β, there are only three amino acid differences of which two are highly conservative (see Fig. 3). Thus, methionine-281 is replaced by isoleucine and isoleucine-284 by leucine in β tropomyosin. The third substitution, which involves a charge change, is the replacement of histidine-276 by asparagine. These small differences imply that the kinase enzyme responsible for the phosphorylation must be specific. Alternatively, there is the possibility that *in vivo* both α and β components are phosphorylated and that, even in the presence of phosphatase inhibitors, the β component is rapidly and completely dephosphorylated during its isolation. This implies the presence of a phosphatase with greater specificity for the β component. A comparison of the sequences about the phosphorylation sites of a number of enzymes and proteins including the 18,000 dalton light chain of myosin with that of tropomyosin shows no obvious similarity and is consistent with reports in the literature that tropomyosin is not phosphorylated by either phosphorylase kinase (22) or cAMP-dependent protein kinase (23). An understanding of the enzymic mechanisms by which this phosphorylation and dephosphorylation takes place must await further studies.

At present the physiological significance of the phosphorylation of tropomyosin in frog and rabbit muscles is not known. This is particularly true of the rabbit skeletal tissue in which the low level of phosphorylation observed was not significantly increased by carrying out the extraction in the presence of phosphatase inhibitors. However, in the case of frog skeletal and rabbit cardiac tissues, the increased levels of phosphorylation observed and the finding that only the α component is phosphorylated imply a physiological role. These observations may well be related to differences in the proportions of α and β components in various muscle types and to differences in their activity and control.

Recent experimental evidence (24–26) and theoretical considerations (27) have strongly indicated that the two α-helices of the coiled coil are present in a nonstaggered arrangement. Considerations of the molecular length of the molecule (410 ± 4 Å) estimated from x-ray diffraction studies of crystals of the protein (28) as opposed to lengths estimated from the amino acid sequence (284 × 1.49 Å = 423 Å, where 1.49 Å is the residue translation in a coiled coil) indicate that a molecular overlap of 8 or 9 residues between the NH_2- and COOH-terminal ends of the molecule occurs when tropomyosin molecules polymerize. McLachlan and Stewart (27) have proposed a model for this overlap that would provide a good fit between the broad faces of the two coiled coils and that would be stabilized by a number of nonpolar and ionic interactions between amino acid side chains in the NH_2- and COOH-terminal regions of the two coiled coils. Although this model must be considered as speculative, it is consistent with the observation that removal of several residues including methionine-281 by carboxypeptidase digestion of tropomyosin largely abolishes head-to-tail polymerization as estimated from viscosity measurements (29–31). Inspection of such a model in our own laboratory indicates that the phosphorylation of one serine-283 on one side of the overlap structure could lead to the formation of a salt bridge to the ϵ-NH_2 of lysine-12 while the phosphorylation of the other serine-283 could form a similar salt linkage with lysine-6 on the other side. In this arrangement, the overlap region would be further stabilized by this additional pair of salt linkages on either side of the head-to-tail overlap region. The lack of phosphate in β tropomyosin would, therefore, predict a significant difference in the degree of polymerization between $\alpha\alpha$ and $\alpha\beta$ dimers which appear to be the only dimer forms that exist in tropomyosin preparations (20, 26, 32).

We are indebted to Dr. B. Edwards, Miss L. Lee, and Dr. B. Sykes of the Biochemistry Department at the University of Alberta who carried out the NMR analyses, and to Dr. H. J. Ribolow of the Department of Biological Chemistry at the University of Illinois for some of the frog tropomyosin. We acknowledge the expert technical assistance of Mr. M. Natriss, Mrs. L. Serink, and Mr. J. D. Glowicki. This work was supported by a grant to the Medical Research Council of Canada Group on Protein Structure and Function at the University

of Alberta, by Grant NS-12172 from the National Institutes of Health, and by the Muscular Dystrophy Association, Inc.

1. Smillie, L. B. (1976) *PAABS Revista* **5**; 183–198.
2. Cummins, P. & Perry, S. V. (1973) *Biochem. J.* **133**, 765–777.
3. Hodges, R. S., Sodek, J., Smillie, L. B. & Jurasek, L. (1972) *Cold Spring Harbor Symp. Quant. Biol.* **37**, 299–310.
4. Stone, D., Sodek, J., Johnson, P. & Smillie, L. B. (1975) in *Proceedings of the 9th FEBS Meeting, Proteins of Contractile Systems*, ed. Biro, E. N. A. (Akademiai Kiado, Budapest, and North-Holland Publishing, Amsterdam), Vol. 31, pp. 125–136.
5. Sodek, J., Hodges, R. S. & Smillie, L. B. (1978) *J. Biol. Chem.* **253**, 1129–1136.
6. Stone, D. & Smillie, L. B. (1978) *J. Biol. Chem.* **253**, 1137–1148.
7. Cummins, P. & Perry, S. V. (1974) *Biochem. J.* **141**, 43–49.
8. Leger, J., Bouveret, P., Schwartz, K. & Swynghedauw, B. (1976) *Pflügers Arch.* **362**, 271–277.
9. Perry, S. V. (1976) in *Contractile Systems in Non-Muscle Tissues*, eds. Perry, S. V., Margreth, A. & Adelstein, R. S. (North-Holland Publishing, Amsterdam), pp. 141–151.
10. Ribolow, H. & Bárány, M. (1977) *Arch. Biochem. Biophys.* **179**, 718–720.
11. Hodges, R. S. & Smillie, L. B. (1972) *Can. J. Biochem.* **50**, 312–329.
12. Woods, E. F. (1967) *J. Biol. Chem.* **242**, 2859–2871.
13. Sender, P. M. (1971) *FEBS Lett.* **17**, 106–110.
14. Dreyer, W. J. & Bynum, E. (1967) in *Methods in Enzymology*, ed. Hirs, C. H. W. (Academic, New York), Vol. 11, pp. 32–39.
15. Heilmann, J., Barollier, J. & Watzke, E. (1957) *Hoppe-Seyler's Z. Physiol. Chem.* **309**, 219–220.
16. Stull, J. T. & Buss, J. E. (1977) *J. Biol. Chem.* **252**, 851–857.
17. Hartley, B. S. (1970) *Biochem. J.* **119**, 805–822.
18. Pearlstone, J. R., Carpenter, M. R., Johnson, P. & Smillie, L. B. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1902–1906.
19. Ookubo, N. (1977) *J. Biochem. (Tokyo)* **81**, 923–931.
20. Offord, R. E. (1966) *Nature* **211**, 591–593.
21. Houmard, J. & Drapeau, G. R. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 3506–3509.
22. Perry, S. V. & Cole, H. A. (1973) *Biochem. J.* **131**, 425–428.
23. Pratje, E. & Heilmeyer, L. M. G. (1972) *FEBS Lett.* **27**, 89–93.
24. Johnson, P. & Smillie, L. B. (1975) *Biochem. Biophys. Res. Commun.* **64**, 1316–1322.
25. Stewart, M. (1975) *FEBS Lett.* **53**, 5–7.
26. Lehrer, S. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3377–3381.
27. McLachlan, A. D. & Stewart, M. (1975) *J. Mol. Biol.* **98**, 293–304.
28. Cohen, C., Caspar, D. L. D., Parry, D. A. D. & Lucas, R. M. (1971) *Cold Spring Harbor Symp. Quant. Biol.* **36**, 205–216.
29. Tawada, Y., Ohara, H., Ooi, T. & Tawada, K. (1975) *J. Biochem. (Tokyo)* **78**, 65–72.
30. Ueno, H., Tawada, Y. & Ooi, T. (1976) *J. Biochem. (Tokyo)* **80**, 283–290.
31. Johnson, P. & Smillie, L. B. (1977) *Biochemistry* **16**, 2264–2269.
32. Yamaguchi, M., Greaser, M. L. & Bassens, R. G. (1974) *J. Ultrastruct. Res.* **48**, 33–58.