# NON-POLYMERIZABILITY OF PLATELET TROPOMYOSIN AND ITS $NH_2$- AND COOH-TERMINAL SEQUENCES

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## 1. Introduction

Tropomyosin molecules from skeletal and cardiac muscle are highly asymmetric structures in which the two constituent polypeptide chains are arranged as α-helices in a coiled-coil structure. At low ionic strength these molecules aggregate head-to-tail to form extended filaments which in vivo lie in the two grooves of the F-actin structure. The binding of calcium to the troponin-C component of the troponin complex, with which each tropomyosin molecule is associated, leads to a change in the position of the tropomyosin in the actin grooves and to the interaction of myosin heads and actin [1].

Tropomyosin from rabbit skeletal muscle is composed of two major forms, α and β, which have identical molecular lengths but show 39 differences in their amino acid sequences [2]. These two forms are separable under a variety of conditions of SDS–polyacrylamide gel electrophoresis (PAGE) or by chromatography on CM-cellulose, at pH 4.0, in 8 M urea [3–5]. They are partially separated by chromatography under non-denaturing conditions on hydroxylapatite [6,7].

Tropomyosin-like molecules can be isolated from a variety of non-muscle tissues and although as yet poorly characterized, appear to be similar to muscle tropomyosin in terms of amino acid composition, the formation of paracrystals in the presence of divalent cations and their ability to confer calcium sensitivity, in the presence of troponin, on actomyosin interactions [8–11]. The non-muscle tropomyosins, however, are $\lesssim 1/7$th their muscle counterparts with a subunit mol. wt ~29 000. They also show at least 10 peptide map differences from the muscle tropomyosins [11].

We have isolated tropomyosin from horse platelets and shown that the purified protein has an α-helical content and thermal stability very similar to that of skeletal tropomyosin. The protein is separable into two bands on SDS–polyacrylamide gels and these are partially separated by chromatography on hydroxylapatite. However, the ability of the platelet tropomyosin to form end-to-end aggregates at low ionic strength as judged from viscosity and molecular weight determinations is markedly reduced when compared with muscle tropomyosin. This lack of aggregation is correlated with markedly different $NH_2$- and COOH-terminal sequences.

## 2. Materials and methods

Tropomyosin was purified from freshly isolated horse platelets by successive homogenizations in a Waring blender with 0.1 M NaCl and 1.0 M NaCl buffered with 10 mM Tris–HCl, 2.5 mM EDTA, at pH 8.0. Following several isoelectric precipitations, at pH 4.6, the protein was purified to better than 95% homogeneity by ion-exchange chromatography on aminohexyl-Sepharose and hydroxylapatite. Full details will be published elsewhere. The purity of the product was monitored by SDS–polyacrylamide gel electrophoresis (PAGE) either as described earlier in phosphate buffer [12] or by the

Laemmli procedure [13] in Tris buffer. Circular dichroism spectra and melting data were collected as in [14]. Protein concentrations were determined by amino acid analysis assuming a value of 29 alanine residues/platelet tropomyosin polypeptide chain. Viscosity experiments were performed using a Cannon-Manning semi-micro viscometer, type A50, with a flow-through time for water of about 5 min. All solutions were dialyzed against the appropriate buffer overnight and rendered dust-free by passage through a 10–20 μm sintered glass filter. Molecular weights were determined by sedimentation equilibrium analysis in 0.1 M NaCl, 20 mM phosphate, 1 mM dithiothreitol, pH 7.0 buffer. The $NH_2$-terminal peptide derived from a tryptic digest was recognized by its failure to yield a dansyl derivative after reaction with dansyl-chloride and acid hydrolysis. It was isolated in pure form by chromatography on Chromobeads type P resin followed by high-voltage electrophoresis, at pH 6.5 [15,16]. Under the latter condition the peptide was neutral. Its partial sequence was elucidated by application of the dansyl-Edman method to peptides derived from *Armillaria mellea* protease [17] and *S. aureus* protease digestion [18] as well as partial acid hydrolysis [19]. The intact peptide was also digested with penicillocarboxypeptidase S [20]. For COOH-terminal analysis of the intact protein, carboxypeptidase Y (Pierce) digestions were performed at 36°C in 0.1 M pyridine acetate buffer, pH 5.9, containing 60 μM norleucine [21]. The enzyme: protein ratio was 1:200 with a platelet tropomyosin concentration of 2 mg/ml. Following termination of the reaction by heating in a boiling water bath for 5 min, the precipitate was removed by centrifugation and free amino acids in the supernatant analyzed on a Durrum D-500 amino acid analyzer. Values for each digestion time were standardized using norleucine. The precipitated protein was subjected to SDS–PAGE to ensure that no endopeptidase cleavages had occurred.

Fig.1a

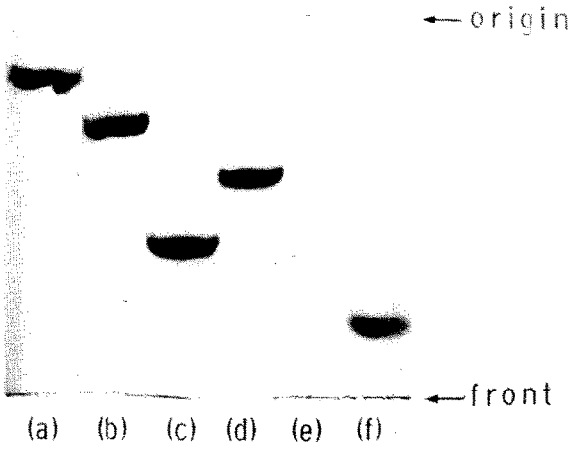
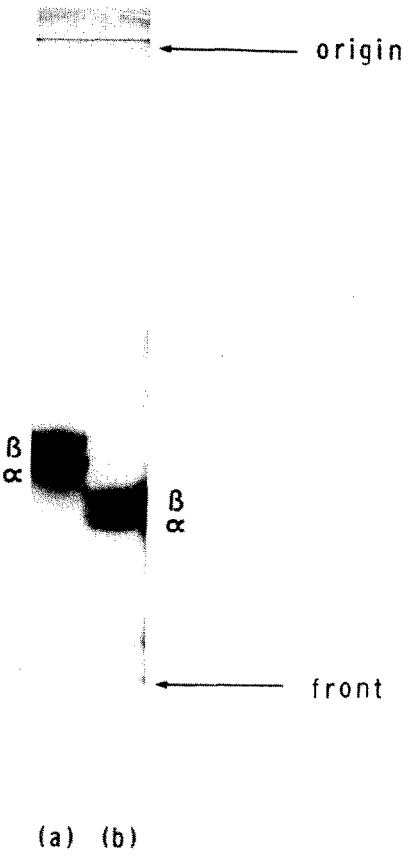



Fig.1a. SDS–polyacrylamide gel electrophoresis on 5% gels buffered with phosphate, pH 7.0 as in [12]. Platelet tropomyosin runs as a single band on this system: (a) phosphorylase, $M_r$ 93 000; (b) bovine serum albumin, $M_r$ 67 000; (c) horse platelet tropomyosin; (d) ovalbumin, $M_r$ 45 000; (e) chymotrypsinogen, $M_r$ 26 000; (f) ribonuclease, $M_r$ 12 600. Fig.1b. SDS–polyacrylamide gel electrophoresis on 8% gel buffered with Tris–HCl as in [13]: (a) rabbit skeletal tropomyosin; (b) horse platelet tropomyosin.

Fig.1b



## 3. Results

After purification on a hydroxylapatite column the platelet tropomyosin runs as a single band on SDS–PAGE–phosphate gels with subunit mol. wt 28 000 (fig.1a). On SDS–PAGE–Tris gels [13], the platelet tropomyosin is seen to consist of two bands (fig.1b) with the slower moving band generally forming a higher percentage of the total protein. In conformity with the nomenclature adopted for the muscle tropomyosins the faster component is designated α and the slower β. The amino acid composition of the horse platelet tropomyosin was found to be virtually identical to the human platelet protein isolated [8]. The only significant difference was that cysteine, determined as cysteic acid after performic acid oxidation [22], was present as 2 residues/chain.

The platelet molecule, although shorter, appears to form as stable a coiled-coil as the muscle molecule. Thermal denaturation curves (fig.2) using the circular dichroism ellipticity at 222 nm as a measure of helical content, indicated a melting temperature ($T_m$) in 0.1 M KCl, 20 mM phosphate, 1 mM dithiothreitol, pH 7.0 of 47°C and in 1.0 M KCl, 20 mM phosphate, 1 mM dithiothreitol, pH 7.0, of 51°C. Under identical conditions skeletal α tropomyosin had $T_m$ values of 44°C and 51°C, respectively. Calculations of helical content at 20°C from the ellipticity at 210 nm and 222 nm gave values greater than 95% [23].

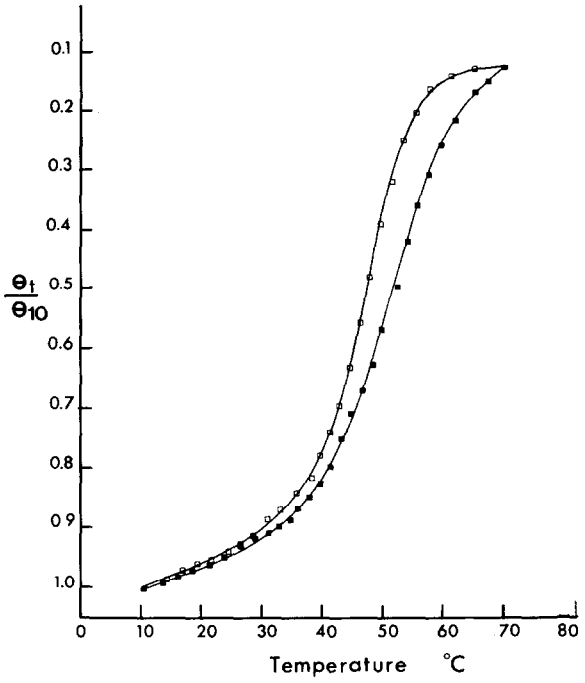



Fig.2. Circular dichroism thermal denaturation curves of platelet tropomyosin in 1.0 M NaCl (□) and 0.1 M NaCl (■) 50 mM $PO_4$, 1 mM DTT, pH 7.0. The ratio $\theta_t/\theta_{10}$ is the fractional ellipticity observed at the indicated temperature with respect to the ellipticity at 10°C, at 222 nm.

An important property of the muscle tropomyosins is their ability to aggregate head-to-tail at low ionic strength, a phenomenon which results in a large increase in viscosity [24,25]. As indicated in fig.3, the relative viscosity of rabbit skeletal α tropomyosin increases sharply as the ionic strength is lowered below 0.1. Platelet tropomyosin, however, shows a much smaller viscosity increase indicating a decreased tendency for end-to-end aggregation. Further evidence for a decreased ability of the platelet tropomyosin to aggregate was obtained by sedimentation equilibrium measurements in 0.1 M KCl, 0.01 M Tris–HCl, pH 8.0. Under these conditions α tropomyosin was present largely as a

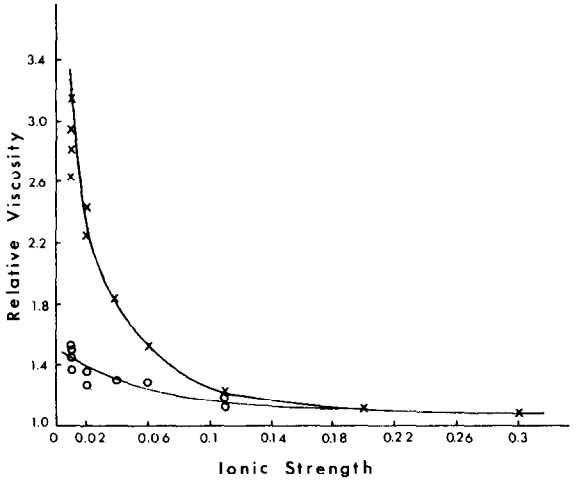



Fig.3. Relative viscosity versus ionic strength curves for rabbit skeletal α tropomyosin (×) and horse platelet tropomyosin (○) at 2 mg/ml. Concentrations were adjusted by using the $A_{280}$. $E^{1\%}_{280}$ was taken as 3.3 for skeletal α tropomyosin, and 2.8 for platelet tropomyosin. All solutions contained 2 mM mercaptoethanol, 10 mM cacodylate pH 7.0. Each sample was dialyzed overnight against the appropriate buffer before reading the viscosity.

$NH_2$-terminal sequence

| | 1 5 10 |
|---|---|
| Horse platelet | X-(Leu,Gly,Ala)-Asn-Ser-Leu-Glu-Ala-Val-Lys-Arg- |
| Rabbit skeletal α | Ac-Met-Asp-Ala - Ile-Lys-Lys-Lys-Met-Gln-Met-Leu- |

COOH-terminal sequence

| | 280 284 |
|---|---|
| Horse platelet | -Ser-Glu-Leu-Leu-Ile-$COO^-$ |
| Rabbit skeletal α | -Asp-Met-Thr-Ser-Ile-$COO^-$ |

Fig.4. Comparison of $NH_2$- and COOH-terminal sequences of horse platelet and rabbit skeletal tropomyosins. Numbering of residues is for the rabbit skeletal muscle protein [15,16].

mixture of dimers and trimers with mol. wt 140 000–160 000, while the platelet tropomyosin gave values of 50 000–90 000 indicating monomers in equilibrium with some dimeric material.

These differences in head-to-tail aggregation suggested that either one or both of the $NH_2$- and COOH-terminal sequences of the platelet tropomyosin were significantly different from the muscle protein. This was confirmed by the isolation and partial sequence determination of a blocked $NH_2$-terminal peptide from a tryptic digest of the citraconylated protein. A tentative sequence for the COOH-terminus of the protein was elucidated by treatment of the intact protein with carboxypeptidase Y. A comparison of these sequences with those of the rabbit skeletal α component (fig.4) shows no homology at the $NH_2$-terminus and significant differences at the COOH-terminus.

## 4. Discussion

Platelet tropomyosin is shown to consist of two types of subunits which can be resolved on SDS–PAGE in the Laemmli discontinuous buffer system [13]. The two forms are probably highly homologous as is the case for the α and β forms of rabbit skeletal muscle tissue [2,15,16]. Recently it has been shown that brain tropomyosin also consists of two different polypeptide chains [26] and this may be a common feature of tropomyosins from all non-muscle cells. Although separable on SDS–PAGE under certain conditions there is no reason to believe that they have significantly different molecular weights. The α and β components of rabbit skeletal tropomyosin are now known to be identical in molecular length [2].

Surprisingly the platelet tropomyosin has significantly less tendency to form end-to-end aggregates than muscle tropomyosin as indicated by lower viscosity and molecular weight measurements at low ionic strength. The removal of 4 residues including Met-281 from the COOH-terminus of skeletal muscle α-tropomyosin with carboxypeptidase A has lead to a loss of polymerizability [12,27,28]. The chemical modification of Lys-7 and one or more of the methionines in the $NH_2$-terminal region has a similar effect [12]. The finding in the present work that the $NH_2$-terminal and COOH-terminal sequences of platelet tropomyosin are markedly different from the muscle protein, even though other regions of the sequence show a higher degree of similarity (W. G. L., G. and L. B. S., unpublished), therefore accounts for its relative inability to form head-to-tail aggregates at low ionic strength.

The significance of these observations in terms of the possible role of platelet tropomyosin in the

regulation of actomyosin interaction is presently unclear. Non-polymerizable skeletal muscle tropomyosin, prepared by treatment with carboxypeptidase A, in combination with troponin has been reported to repress the superprecipitation of actomyosin in the absence of calcium, while this repression was released by the addition of calcium [27]. However, the curve representing the superprecipitation rate as a function of pCa was less steep than that found with untreated tropomyosin, troponin and actomyosin. These results indicate that a degree of cooperativity in the regulation of muscle contraction is mediated by head-to-tail interaction of tropomyosin molecules along the thin filament. Since this head-to-tail interaction in platelet tropomyosin appears to be less extensive and perhaps lacking, it can be predicted that the platelet tropomyosin will show a lesser degree of cooperativity in the calcium regulation of a reconstituted muscle actomyosin system.

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## References

[1] For recent reviews see: Cohen, C. (1975) Sci. Am. 233, 36–45; Mannherz, H. G. and Goody, R. S. (1976) Ann. Rev. Biochem. 45, 427–465; Smillie, L. B. (1976) PAABS Revista 5, 183–198; Squire, J. M. (1975) Ann. Rev. Biophys. Bioeng. 4, 137–163.
[2] Mak, A. and Smillie, L. B., unpublished.
[3] Sender, P. M. (1971) FEBS Lett. 17, 106–110.
[4] Cummins, P. and Perry, S. V. (1973) Biochem. J. 133, 765–777.
[5] Strasburg, G. M. and Greaser, M. L. (1976) FEBS Lett. 72, 11–14.
[6] Eisenberg, E. and Kielley, W. Y. (1974) J. Biol. Chem. 249, 4742–4748.
[7] Yamaguchi, M., Greaser, M. L. and Cassens, R. G. (1974) J. Ultrastruc. Res. 48, 33–58.
[8] Cohen, I. and Cohen, C. (1972) J. Mol. Biol. 68, 383–387.
[9] Fine, R. E., Blitz, A. L., Hitchcock, S. E. and Kaminer, B. (1973) Nature New Biol. 245, 182–185.
[10] Fine, R. E. and Blitz, A. L. (1975) J. Mol. Biol. 95, 447–454.
[11] Mahendran, C. and Berl, S. (1977) Proc. Natl. Acad. Sci. USA 74, 2273–2277.
[12] Johnson, P. and Smillie, L. B. (1977) Biochemistry 16, 2264–2269.
[13] Laemmli, U. K. (1970) Nature 227, 680–685.
[14] Pearlstone, J. and Smillie, L. B. (1977) Can. J. Biochem. 55, 1032–1038.
[15] Sodek, J., Hodges, R. S. and Smillie, L. B. (1978) J. Biol. Chem. 253, 1129–1136.
[16] Stone, D. and Smillie, L. B. (1978) J. Biol. Chem. 253, 1137–1148.
[17] Lewis, W. G., Basford, J. M. and Walton, P. L. (1978) Biochim. Biophys. Acta 522, 551–560.
[18] Houmard, J. and Drapeau, G. R. (1972) Proc. Natl. Acad. Sci. USA 69, 3506–3509.
[19] Hodges, R. S. and Smillie, L. B. (1972) Can. J. Biochem. 50, 312–329.
[20] Jones, S: R. and Hoffmann, T. (1972) Can. J. Biochem. 50, 1297–1310.
[21] Hayashi, R., Moore, S. and Stein, W. H. (1973) J. Biol. Chem. 248, 2296–2302.
[22] Moore, S. (1963) J. Biol. Chem. 238, 235–237.
[23] Chen, Y. H., Yang, J. T. and Chau, K. H. (1974) Biochemistry 13, 3350–3359.
[24] Tsao, T. C., Bailey, K. and Adair, G. S. (1951) Biochem. J. 49, 27–36.
[25] McCubbin, W. D., Kouba, R. F. and Kay, C. M. (1967) Biochemistry 6, 2417–2425.
[26] Bretscher, A. and Weber, K. (1978) FEBS Lett. 85, 145–148.
[27] Tawada, Y., Ohara, H., Ooi, T. and Tawada, K. (1975) J. Biochem. (Japan) 78, 65–72.
[28] Ueno, H., Tawada, Y. and Ooi, T. (1976) J. Biochem. (Japan) 80, 283–290.