PLATELET TROPOMYOSIN: LACK OF BINDING TO SKELETAL MUSCLE TROPONIN AND CORRELATION WITH SEQUENCE

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1. Introduction

Control of contraction in skeletal and cardiac muscle tissues is mediated by the troponin complex composed of troponin-T (Tn-T) which binds to tropomyosin, troponin-C (Tn-C) which binds calcium ions and troponin-I (Tn-I) which inhibits the magnesium-stimulated actomyosin ATPase. The binding of calcium to the Tn-C component is known to bring about a conformational change in this subunit which is believed to be transmitted through the other members of the troponin complex to tropomyosin. Tropomyosin changes its position in the grooves of the double stranded F-actin helix allowing myosin heads to interact with actin.

Contractile proteins similar to those found in muscle perform many vital functions, both structural and motile, in practically all eukaryotic cells [1]. In particular, non-muscle tropomyosin can be isolated from blood platelets, brain, pancreas and fibroblasts [2–5]. These proteins are similar to the muscle tropomyosins in terms of amino acid composition, α-helical content and ability to bind to actin and form paracrystals. However, they appear to be smaller molecules than the muscle tropomyosins (29 000 daltons/polypeptide chain as compared with 33 000 daltons).

A non-muscle tropomyosin isolated from horse platelets was found to differ from the rabbit skeletal muscle molecule in its ability to form end-to-end aggregates and in its NH_2- and COOH-terminal sequences [6]. In the present report we show that the amino acid sequence of the platelet protein is very similar to the muscle α- and β-tropomyosins in the region of the hypothetical troponin binding site. In spite of this the platelet molecule does not bind to rabbit skeletal muscle troponin.

2. Materials and methods

Tropomyosin was purified from horse platelets as before [6]. Following carboxymethylation with iodoacetic acid, 120 mg (4 μmol) was dissolved in 15 ml 50 mM phosphate buffer, pH 8.0 and citraconylated with a 40-fold molar excess of citraconic anhydride over total lysine content. Following desalting on Sephadex G-25 the freeze-dried product was dissolved in 0.2 M NH_4HCO_3, pH 8.0 and digested with 1.2 mg TPCK-trypsin (Worthington) at 37°C for 2 h. The reaction was terminated with 1 mM diisopropylfluorophosphate and the product lyophilized. Following decitraconylation in 5% formic acid, the sample was applied to a 200 × 2.5 cm Sephadex G-75 column equilibrated with 5% formic acid. The column effluent was monitored by ninhydrin analyses after alkaline hydrolysis and by A_{280} measurements. The first peak eluted from the column represented a homogeneous peptide as determined by its NH_2-terminal sequence and its migration as a single band on high-voltage paper electrophoresis, at pH 6.5 (mobility relative to Asp = –0.42). The peptide (750 nmol) was dissolved in 4 ml 0.2 M NH_4HCO_3, pH 8.0 and digested with TPCK-trypsin at a 100:1 molar ratio of peptide: enzyme for 5 h at 37°C. Following termination of the digestion by freeze-drying the resulting peptides were fractionated by high-voltage paper electrophoresis, at pH 6.5, 3.5 and/or 1.8. Methods for the latter

procedures, amino acid analyses, manual sequence analysis and assignment of amides are in [7,8]. The intact peptide (200 nmol) was also subjected to automatic sequence analysis in a Beckman Model 890B sequencer as in [9]. Parts of the sequence were confirmed by analysis of peptides derived from *Staphylococcus aureus* protease digestion [10].

Viscosity measurements of intact rabbit skeletal α- and platelet tropomyosins were performed using a Cannon-Manning semi-micro viscometer, type A50, with a flow-through time for water of about 5 min. Tropomyosin and troponin solutions were dialyzed overnight and for 2 h, respectively, against the buffer (0.1 M NaCl, 10 mM cacodylate, 2.0 mM mercaptoethanol, pH 7.0) at 4°C. Protein concentrations were determined by $A_{280\ nm}$ using $E^{1\%}_{280\ nm}$ as 3.3 for skeletal tropomyosin [11], 4.5 for skeletal troponin [12] and 2.8 for platelet tropomyosin [6]. Solutions of mixtures of tropomyosin and troponin at various molar ratios were allowed to stand for 2 h before the viscosities were read at 20°C.

Affinity chromatography experiments were performed on a column of Tn-T coupled to cyanogen bromide-activated Sepharose as in [13].

3. Results

Only one large fragment (TA) is produced by tryptic digestion of citraconylated and carboxymethylated platelet tropomyosin. It can be separated from the other smaller fragments (TB and TC) by gel filtration on a Sephadex G-75 column (fig.1). Amino acid analysis showed that it was very similar to a peptide that can be produced by tryptic digestion of citraconylated rabbit skeletal muscle α- or β-tropomyosin. This fragment encompasses the region from Ala-183 to Arg-238 in the muscle tropomyosin sequences. The sequence of the first 10 residues of the fragment was determined on the automatic sequencer, leaving the majority of the sequence to be established by manual methods applied to the tryptic peptides as in section 2. All acids and amides could be assigned unambiguously from the electrophoretic mobilities of the peptides, at pH 6.5. Overlaps for the tryptic peptides were not determined in all cases but since the homology with the muscle proteins is so striking, the order of the peptides could be assigned with confidence based on the known sequences of the rabbit skeletal muscle tropomyosins. The assigned sequence is illustrated in fig.2 using the numbering system for the muscle proteins.

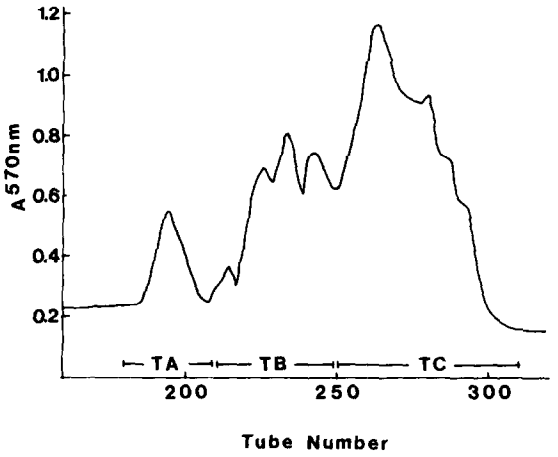

Fig.1. Fractionation of tryptic digest of citraconylated platelet tropomyosin (120 mg) on a Sephadex G-75 column (2.5 × 200 cm) equilibrated with 5% formic acid. Peak TA was pooled as indicated and subjected to sequence analysis as in section 2.

The interaction of platelet tropomyosin with skeletal muscle troponin was studied by observing the effect that the addition of troponin had on the viscosity of a platelet tropomyosin solution. The large increase in viscosity that occurs when troponin is added to skeletal muscle tropomyosin is attributable to the interaction of the two proteins and the promotion of tropomyosin aggregation [14–16]. However, as shown in fig.3, platelet tropomyosin completely lacks this property.

Platelet and skeletal muscle α-tropomyosin were also compared with respect to their relative binding affinities to Tn-T immobilized on Sepharose columns [13]. Platelet tropomyosin was eluted at KCl 0.05 M, a value indistinguishable from that characteristic of the non-specific binding of other coiled-coil proteins such as myosin subfragment 2. Rabbit skeletal α- and β-tropomyosins under the same conditions were eluted at 0.20 M and 0.16 M KCl, respectively. The same results were obtained irrespective of whether Tn-T or whole troponin was used for the preparation of the affinity column.

	183	184	185	186	187	188	189	190	191	192	193	194
Muscle α Tm	Ala	Glu	Leu	Ser	Glu	Gly	Lys	Cys	Ala	Glu	Leu	Glu
Muscle β Tm	—	—	Val	Ala	—	Ser	—	—	Gly	Asp	—	—
Platelet Tm	—	—	Val	—	—	Leu	—	—	Gly	Asp	—	—

	195	196	197	198	199	200	201	202	203	204	205	206
Muscle α Tm	Glu	Glu	Leu	Lys	Thr	Val	Thr	Asn	Asn	Leu	Lys	Ser
Muscle β Tm	—	—	—	—	Ile	—	—	—	—	—	—	—
Platelet Tm	—	—	—	—	Asn	—	—	—	—	—	—	—

	207	208	209	210	211	212	213	214	215	216	217	218
Muscle α Tm	Leu	Glu	Ala	Gln	Ala	Glu	Lys	Tyr	Ser	Gln	Lys	Glu
Muscle β Tm	—	—	—	—	—	Asp	—	—	—	Thr	—	—
Platelet Tm	—	—	—	Ala	Ser	—	—	—	—	Glu	—	—

	219	220	221	222	223	224	225	226	227	228	229	230
Muscle α Tm	Asp	Lys	Tyr	Glu	Glu	Glu	Ile	Lys	Val	Leu	Ser	Asp
Muscle β Tm	—	—	—	—	—	—	—	—	Leu	—	Glu	Glu
Platelet Tm	—	—	—	—	—	—	—	—	Leu	—	—	—

	231	232	233	234	235	236	237	238
Muscle α Tm	Lys	Leu	Lys	Glu	Ala	Glu	Thr	Arg
Muscle β Tm	—	—	—	—	—	—	—	—
Platelet Tm	—	—	—	—	—	—	—	—

Fig.2. Comparison of amino acid sequences of rabbit skeletal muscle α- and β-tropomyosins and horse platelet tropomyosin. Numbering scheme is for the muscle proteins [17,18]. Residues which are identical to those of α-tropomyosin are represented by (——). The hypothetical troponin binding region is boxed.

4. Discussion

In this paper we report the first extensive sequence information on a non-muscle tropomyosin. The reported sequence is for a fragment from horse platelet tropomyosin homologous to the region encompassing residues 183–238 in skeletal α- and β-tropomyosins. In these 56 residues the α- and β-components of the muscle proteins differ from one another at 11 positions [17,18]. Over this same region the platelet tropomyosin differs from that of muscle α-tropomyosin at 9 positions, from that of muscle β-tropomyosin at 9 positions and from both of them at only 5 positions. This remarkable homology must result from the constraints imposed on the tropomyosin sequence by the necessity of retaining its functionally important actin-binding sites [19] as well as the repeating patterns of residues required for the stabilization of its coiled-coil structure [20].

This region of platelet tropomyosin is of particular interest since it contains that portion of the structure of rabbit skeletal muscle tropomyosin (residues 197–217) postulated to be the binding site for Tn-T (see [21] for a summary of the evidence on which this hypothesis is based). Comparison of the amino acid sequences shows that the platelet tropomyosin differs only in three amino acids from both the muscle α- and β-proteins over this region of sequence (see residues

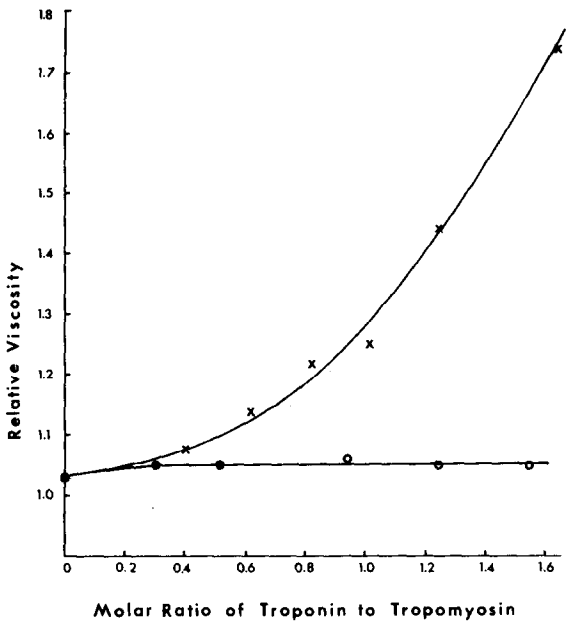

Fig.3. Effect of the addition of rabbit skeletal troponin on the relative viscosities of rabbit skeletal α- (—x—x—) and horse platelet (—o—o—) tropomyosins; tropomyosin was 0.5 mg/ml in 10 mM cacodylate buffer, 0.1 M NaCl, 2.0 mM mercaptoethanol, pH 7.0.

197–217 of fig.2). In only one of these (residue 216), in which Gln in α and Thr in β are changed to Glu in the platelet protein, can the alteration be considered as significant in possibly altering the interaction properties of the molecules with troponin. It is therefore indeed surprising that the platelet protein shows little or no affinity for Tn-T or troponin as shown in the present work both by the absence of a viscosity increase when the components are mixed and by the low affinity of the platelet protein to a Tn-T Sepharose affinity column when compared with the muscle α- and β-proteins.

These observations therefore raise a question as to the validity of the assignment of the Tn-T binding site on skeletal muscle tropomyosin to a position about one-third of the distance from its COOH-terminal end. The evidence for this assignment is in our view not unequivocal and consideration must be given to the possibility that the binding sites for Tn-T on the skeletal muscle tropomyosins are closer to the COOH-termini of the molecules and may even possibly involve the head-to-tail overlap regions of tropomyosin aggregates. Since these regions are markedly altered in platelet tropomyosin as shown [6] this interpretation would be consistent both with the primary structural data as well as with the different interaction properties of the platelet tropomyosin. It would also be consistent with the relative affinities to Tn-T Sepharose affinity columns of a variety of large fragments of skeletal muscle α-tropomyosin [13] and the recent demonstration in our laboratory that the affinity of mixtures of two such fragments to Tn-T is enhanced when one of the fragments has an intact COOH-terminal sequence and the other has an intact NH_2-terminal sequence (M.D.P. and L.B.S., unpublished). However, this evidence is not conclusive and the possibility remains that the binding of Tn-T has been dramatically altered by the few amino acid substitutions observed in the platelet tropomyosin in the region 197–217. One would predict on the basis of the above observations that platelet and perhaps other non-muscle tropomyosins would be relatively ineffective as replacements for skeletal muscle tropomyosin in the calcium regulation of a skeletal muscle troponin–tropomyosin–actomyosin system. An earlier report [5] that brain tropomyosin is at least partially effective in such a system must now be further investigated.

The present results do not of course mean that tropomyosin does not participate as a component of a calcium-sensitive actin-linked regulatory system in non-muscle cells. They do imply that any other regulatory proteins will show substantially different properties from those of the more familiar components of the muscle troponin system.

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