Amino acid sequence of equine platelet tropomyosin

Correlation with interaction properties

William G. Lewis[+], Graham P. Cote[°], Alan S. Mak[†] and Lawrence B. Smillie[*]

Medical Research Council Group in Protein Structure and Function, Department of Biochemistry, University of Alberta, Edmonton, T6G 2H7 Canada

Received 18 March 1983

Equine platelet β tropomyosin (247 residues), like rabbit skeletal muscle α tropomyosin (284 residues) has a repeating pattern of amino acid residues characterisitic of a coiled-coil structure. When compared with the muscle protein, it is extended by 5 residues at the NH_2-terminus and possesses two 21 residue deletions (positions 23–43 and 60–80 of the muscle sequence). The two proteins are highly conserved from residues 81–260, but are significantly different at their COOH-termini (residues 261–284). These differences in platelet tropomyosin can be correlated with its diminshed head-to-tail polymerization, a weaker interaction with F-actin and a reduced affinity for muscle troponin and the T1 fragment of troponin-T.

Coiled-coil *Contractile protein* *Non-muscle* *Actin-binding* *Ca^{2+} regulation* *Troponin interaction*

1. INTRODUCTION

Tropomyosins of skeletal and cardiac tissues are intimately involved, along with the troponin (Tn) complex, in the thin-filament-linked regulatory system for calcium control of actomyosin interaction (reviews [1,2]). Each tropomyosin (TM) molecule is rod-like in shape, 41 nm long, and composed of two highly α-helical subunits wrapped around each other to form a coiled-coil. Various periodic and aperiodic features of its amino acid sequence have been correlated with the stabilization of its coiled-coil in a non-staggered parallel structure, with its head-to-tail aggregation into long molecular filaments and with its interactions with F-actin and Tn (review [3]).

TM's have also been identified in a number of non-muscle tissues, including platelets, brain, pancreas, fibroblasts, sea urchin eggs and mammalian cultured cells [4–12]. The platelet protein [4,10] has an α-helical content >90% and an amino acid composition similar to that of muscle TM. However, the subunit M_r is $\leqslant$30000, with an axial repeat in its paracrystals of ~34 nm, significantly shorter than that (39.5 nm) observed in paracrystals of muscle TM. Although there have been reports of higher M_r forms of TM in non-muscle cells grown in culture [12,13], those detected in differentiated non-muscle tissues appear to be predominantly of the low-M_r variety and to have very similar peptide maps after digestion with chymotrypsin [7].

Equine platelet TM polymerizes less extensively in head-to-tail manner, binds more weakly to both platelet and muscle F-actin, interacts less strongly with Tn, Tn-T and the T1 fragment (residues 1–158) of Tn-T and is less effective than muscle

* To whom correspondence should be addressed

[+] Present address: Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, England

[°] Present address: Laboratory of Cell Biology, National Heart, Blood and Lung Institute, National Institutes of Health, Bethesda, MD 20205, USA

[†] Present address: Department of Biochemistry, Queen's University, Kingston, Ontario, K7L 3N6 Canada

Abbreviations: TM, tropomyosin; Tn, troponin; SDS, sodium dodecylsulfate

Published by Elsevier Science Publishers B.V.
00145793/83/$3.00

TM in the calcium-regulated actomyosin subfragment 1-Tm–Tn ATPase system [10,14–18]. However, the platelet protein is similar to muscle TM in its interaction with the T2 fragment (residues 159–259) of Tn-T and in the Ca^{2+} sensitivity of this interaction in the presence of Tn-C [18]. To provide further information on the relationships between the structure of platelet TM and its functional properties described above, we undertook the elucidation of its amino acid sequence, the results of which are described here.

2. EXPERIMENTAL

TM, isolated as in [10], migrated as α and β components (molar ratio 1:2) on SDS–polyacrylamide electrophoretic gels in the presence of 6 M urea. Attempts to separate these on a preparative scale were unsuccessful and the amino acid sequence analyses were performed on the mixture. Where more than one sequence was observed corresponding to a particular region of the platelet TM structure, the peptide isolated in greater yield was assumed to have been derived from the β component. For sequence analysis, fragments were generated by cyanogen bromide cleavage and tryptic digestion of the citraconylated proteins. Following their purification and decitraconylation, the larger fragments were further digested with trypsin, thermolysin or *Staphylococcus aureus* protease V8. Manual and automatic sequencing methods have been described [19–22]. Full details of the sequence analysis will be reported elsewhere. Some aspects of this work have been reported in [14,15].

3. RESULTS AND DISCUSSION

The sequence of platelet TM presented in fig.1 represents the results of an analysis of the fragments and peptides generated from a mixture of α and β components in a ratio of about 1:2 in the isolated protein. In spite of this, surprisingly little heterogeneity was detected in the isolated peptides, indicating that the α and β components of the platelet protein are highly similar. Heterogeneity was observed at only two positions (residues 143 and 146) where peptides were isolated in lower yield in which Ile-106 and Met-109 were replaced by Leu and Ile. It must be emphasized,

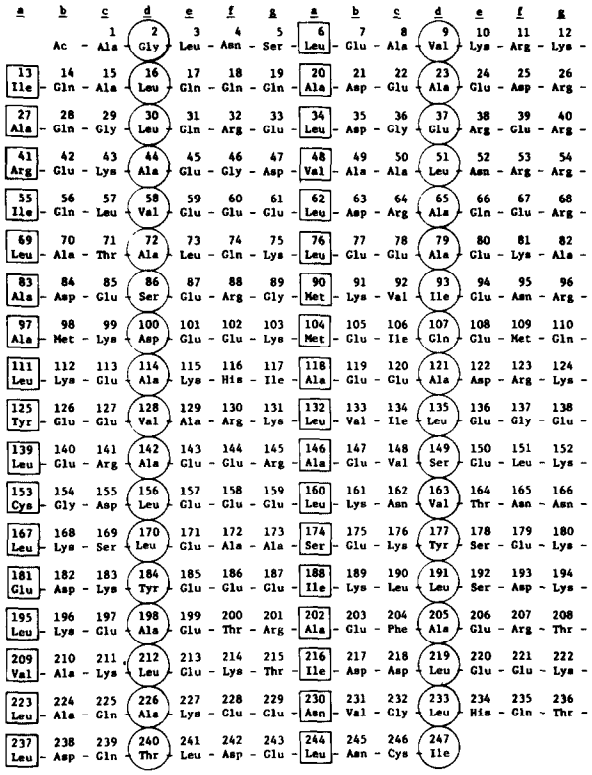

Fig.1. Amino acid sequence of the β-component of equine platelet TM. Sequence is arranged such that a–g positions of the repeating pseudo-heptapeptide, typical of a coiled-coil structure, are vertically aligned. The non-polar core positions a and d are boxed and circled respectively. Heterogeneity was observed at positions 143 and 146, where peptides were recovered in lower yields in which Ile and Met were replaced by Leu and Ile, respectively.

however, that since a number of fragments and peptides isolated in lower yields were not fully characterized in this study, the numbers of substitutions between the α and β components of the platelet protein may be larger than the two indicated above.

One of the striking features of the muscle TM sequence [20] is the repeating pattern of non-polar and polar residues responsible for the stabilization of its coiled-coil structure. As is evident from an inspection of the sequence of the platelet protein (fig.1), this pattern is retained and is continuous from the NH_2-terminus to the COOH-terminus. This is consistent with the known high α-helical content and other properties of the protein as described in [4,10,11]. The a and d positions of the

repeating pseudo-heptapeptide are largely occupied by non-polar residues and only occasionally by polar or charged residues.

For a comparison of platelet TM with the muscle protein, the two sequences have been aligned to maximize homology (fig.2). The platelet protein is seen to be extended at the NH_2-terminal end by 5 residues and to have two 21 residue deletions in the first 80 residues of the muscle protein. Similarity is maximized when residues 6–27 and 28–43 of platelet TM are aligned with residues 1–22 and 44–59 of the muscle protein, respectively. The homology in the first of these alignments (residues 6–27 with 1–22) is very strong, with 9 identical and 6 similar residues out of a total of 22. The second of these alignments is weaker, with 5 identities and 3 similar residues in a total of 16. However, the placement of these regions in any other alignment with the first 80 residues of muscle TM leads to a significantly decreased score level for identities and similarities. The alignment of the remaining sequences is unambiguous, since from residues 81–260 the sequences are highly conserved, and the polypeptide chain lengths to the COOH-terminal ends (residue 284) are identical. Of a total of 180 residues from 81–260, there are only 25 substitutions, and in nearly every case these are highly conservative in nature. From residues 261–284, although there are no deletions or insertions, the platelet sequence differs significantly from the muscle TM structure. This COOH-terminal sequence, as reported here, differs from

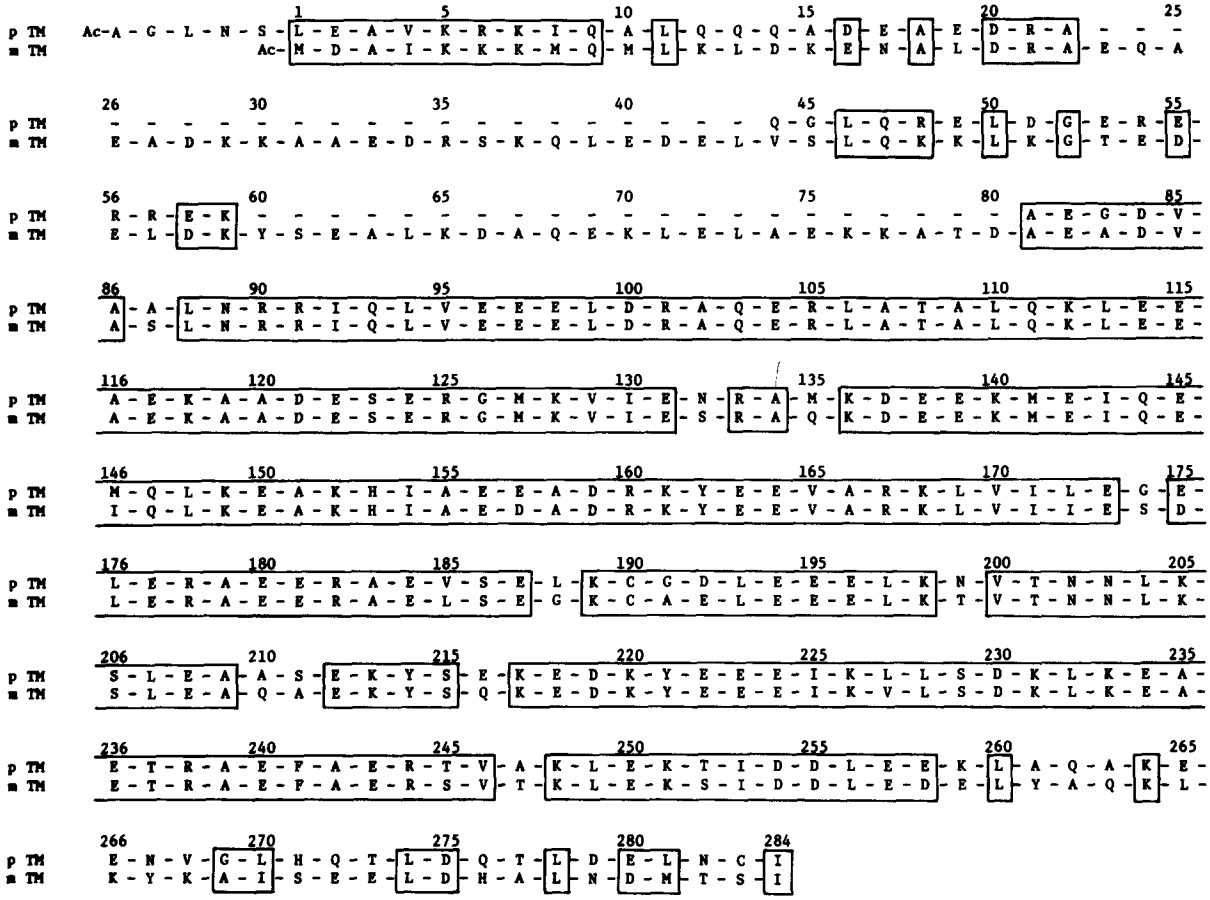

Fig.2. Alignment of equine platelet β- and rabbit skeletal muscle α-TM sequences to maximize homology. The one-letter code for amino acids is used. Regions of identity and similarity are boxed. In this figure, the numbering scheme is that for the muscle protein. Dashes (–) in the platelet sequence are deletions. pTM and mTM are platelet and muscle TMs, respectively. Chemically similar residues are defined here as: R≡K; E≡D; T≡S; G≡A; L≡V≡I≡M.

that in [14]. The latter was a tentative result based only on the results of carboxypeptidase analyses of the intact protein. However, the important conclusion that this region of the platelet sequence differs from muscle TM is corroborated by the present more complete analyses.

The two 21-residue deletions in platelet TM reduce its length by about 1/7 when compared with muscle TM. Thus platelet TM can span only 6 actin monomers on each of the two F-actin strands in agreement with recent qualitative binding studies of the protein to F-actin [15]. In addition to the two deletions there are significant differences in the platelet TM amino acid sequence when compared with residues 1–80 of the muscle TM structure. This suggests that the actin interaction properties of this region of the molecule may differ significantly from those of its muscle counterpart.

Like muscle TM, the interaction of platelet TM with both muscle and platelet F-actin is dependent on the medium [Mg^{2+}] [16]. Whereas the binding of muscle TM reaches a maximum at 1–2 mM Mg^{2+}, that of the platelet protein only reaches saturation when $MgCl_2$ is increased to 8–10 mM and is incomplete at lower, more physiological levels. This weaker interaction can be attributed in part to the shorter length of the molecule with its reduced number of actin binding sites, as well as to changes in the binding sites of the NH_2-terminal domain indicated above. A further important factor affecting the binding of muscle and platelet TMs to F-actin is the extent to which the two proteins polymerize in a head-to-tail manner. It has been shown [23] that the binding of 2 and 3 contiguous TM molecules to F-actin, linked through contacts at their ends, is increased dramatically over that for the binding of an isolated TM molecule. We have shown [14] that platelet TM aggregates in a head-to-tail manner less extensively than muscle TM and this is correlated here with significantly different amino acid sequences at both its NH_2-terminal and COOH-terminal ends. Thus, the strength and cooperativity of platelet TM binding to F-actin is expected to be less than that with muscle TM and is explicable in terms of these structural differences.

A further correlation of the similarities and differences exhibited by the platelet and muscle structures can be made with respect to their interactions with the members of the Tn complex. Recent observations have demonstrated that at least two regions on each of both Tn-T and muscle TM are involved in their mutual interaction [18,24–26]. Thus, the T1 (residues 1–158) and CB1 (residues 1–151) fragments of Tn-T interact with a segment of TM close to or at its COOH-terminal end. This interaction is insensitive to Ca^{2+} in the presence of troponin-C [18]. On the other hand, the T2 region (residues 159–259) of Tn-T interacts with TM in the region of its cysteine-190 residue or ~1/3 of the molecular distance from its COOH-terminal end [27]. This interaction is sensitive to [Ca^{2+}] in the presence of Tn-I and -C, with which T2 also interacts [18,27,28].

In the case of platelet TM, we had shown that the interactions with the troponin complex, with Tn-T and with its T1 and CB1 fragments are significantly weaker than with the muscle protein [15,18]. This is now explicable in terms of the structural differences which exist between the platelet and muscle proteins at their COOH-terminal ends (residues 260–284; see fig.2). Of particular significance in this connection may be the replacement of tyrosine-261 and -267 by alanine and asparagine, respectively. The extent of iodination by lactoperoxidase of these tyrosines, but not other tyrosines, in the muscle protein, is appreciably reduced in the presence of Tn or the CB1 fragment of Tn-T, suggesting that they are involved in the interaction at this site [24]. Their absence in the platelet structure is therefore consistent with a weaker interaction of the platelet protein with the CB1/T1 region of Tn-T.

On the other hand, the T2 region (residues 159–259) of Tn-T has been shown to interact equally well with both platelet and muscle TMs [18]. This is fully consistent with the high degree of homology observed in their structures over an extensive region from residues 81–260 and including those portions of their structures in the neighbourhood of cysteine-190 (see fig.2). Significantly, this interaction between T2 and platelet TM is also sensitive to [Ca^{2+}] in the presence of Tn-C [18,27].

Recently we have demonstrated that Tn-I interacts with muscle TM [28] and that Tn-I will induce the binding of both platelet and muscle TM to F-actin under conditions in which the TMs by themselves do not bind to F-actin [16]. This suggests that the binding sites on both TMs for Tn-I

are highly similar, a deduction consistent with the demonstration that the location of Tn-I, -C and the T2 fragment of Tn-T in the muscle thin-filament assembly are coincident [25] and therefore in the region of cysteine-190 of muscle TM.

These observations have certain implications for the structural and molecular properties of putative Tn-like components in non-muscle cells. They would suggest that, if such proteins do in fact exist, they can be expected to have substantially different molecular and interaction properties than the more familiar muscle Tn components. This would be particularly true for any component(s) which might have a Tn-T-like function in such tissues, since the structural requirements for the binding of the T1 portion of muscle Tn-T to platelet TM are evidently lacking. In addition, the significant alterations in structure in the NH_2-terminal region of the platelet TM suggest that this domain may interact with as yet unidentified protein component(s) in non-muscle tissues. Clearly, the identification and characterization of TM-binding proteins in non-muscle tissues would be a useful experimental approach to such questions, an avenue of investigation we are presently pursuing.

ACKNOWLEDGEMENTS

We are indebted to Dr J.R. Pearlstone for her assistance in the computer analysis of the sequences, and for helpful discussions. We thank Mrs Krystyna Golosinka for expert technical assistance, Mr M. Carpenter for the automated sequence analyses and help with the preparation of platelets, and Mr M. Nattriss for the many amino acid analyses. W.G.L. was a recipient of a Muscular Dystrophy Association of Canada postdoctoral fellowship, and G.P.C. the recipient of a Medical Research Council of Canada studentship. This work was supported by the Medical Research Council of Canada.

REFERENCES

[1] Adelstein, R.S. and Eisenberg, E. (1980) Annu. Rev. Biochem. 49, 921–956.
[2] Taylor, E.W. (1979) CRC Crit. Rev. Biochem. 6, 103–164.
[3] Smillie, L.B. (1979) Trends Biochem. Sci. 4, 151–164.
[4] Cohen, I. and Cohen, C. (1972) J. Mol. Biol. 68, 383–387.
[5] Fine, R.E., Lehman, W., Head, J. and Blitz, A. (1975) Nature 258, 260–262.
[6] Fine, R.E., Blitz, A.L., Hitchcock, S.E. and Kaminer, B. (1973) Nature New Biol. 245, 182–186.
[7] Fine, R.E. and Blitz, A.L. (1975) J. Mol. Biol. 95, 447–454.
[8] Bretscher, A. and Weber, K. (1978) FEBS Lett. 85, 145–148.
[9] Ishimoda-Tokagi, T. (1978) J. Biochem. 83, 1757–1762.
[10] Cote, G.P. and Smillie, L.B. (1981) J. Biol. Chem. 256, 11004–11010.
[11] Der Terrossian, E., Fuller, S.D., Stewart, M. and Weeds, A.G. (1981) J. Mol. Biol. 153, 147–167.
[12] Schloss, J.A. and Goldman, A.D. (1980) J. Cell Biol. 87, 633–642.
[13] Paulin, D., Parreau, J., Jakob, H., Jacob, F. and Yaniv, V. (1979) Proc. Natl. Acad. Sci. USA 76, 1891–1895.
[14] Cote, G.P., Lewis, W.G. and Smillie, L.B. (1978) FEBS Lett. 91, 237–241.
[15] Cote, G.P., Lewis, W.G., Pato, M.D. and Smillie, L.B. (1978) FEBS Lett. 94, 131–135.
[16] Cote, G.P. and Smillie, L.B. (1981) J. Biol. Chem. 256, 7257–7261.
[17] Cote, G.P. and Smillie, L.B. (1981) J. Biol. Chem. 256, 11999–12004.
[18] Pearlstone, J.R. and Smillie, L.B. (1982) J. Biol. Chem. 257, 10587–10592.
[19] Pearlstone, J.R., Johnson, P., Carpenter, M.R. and Smillie, L.B. (1977) J. Biol. Chem. 252, 983–989.
[20] Stone, D. and Smillie, L.B. (1978) J. Biol. Chem. 253, 1137–1148.
[21] Mak, A.S., Smillie, L.B. and Stewart, G.R. (1980) J. Biol. Chem. 255, 3647–3655.
[22] Lewis, W.G. and Smillie, L.B. (1980) J. Biol. Chem. 255, 6854–6859.
[23] Walsh, T.P. and Wegner, A. (1980) Biochim. Biophys. Acta 626, 79–87.
[24] Mak, A.S. and Smillie, L.B. (1981) J. Mol. Biol. 149, 541–550.
[25] Ohtsuki, I. (1979) J. Biochem. (Tokyo) 86, 491–497.
[26] Katayama, E. (1979) J. Biochem. (Tokyo) 85, 1379–1381.
[27] Chong, P.C.S. and Hodges, R.S. (1982) J. Biol. Chem. 257, 9152–9160.
[28] Pearlstone, J.R. and Smillie, L.B. (1983) J. Biol. Chem. 258, 2534–2542.