# CHEMICAL EVIDENCE FOR CHAIN HETEROGENEITY IN RABBIT MUSCLE TROPOMYOSIN

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Amino acid analyses of tropomyosin have shown 4 histidines, 13-14 methionines and close to 3 cysteine residues per mole (M.Wt. 70,000) of tropomyosin. The isolation of 2 unique histidyl and 5 unique methionyl peptides is consistent with two identical polypeptide chains of molecular weight 35,000. However, the presence of 3 cysteines and 3 unique cysteine sequences per mole of 70,000 is indicative of either non-identical but similar chains or of more than one form of tropomyosin. This conclusion is further substantiated by the isolation and sequence analysis of two varieties of peptides arising from the C-terminus of the protein. The previous report of an N-acetylated peptide has been confirmed and its sequence elucidated. The limited sequences reported indicate a regular repeat of hydrophobic residues as required by the inter-chain packing of a coiled-coil structure.

Physical studies of rabbit skeletal tropomyosin have shown that it is a rod-like molecule of molecular weight about 70,000 and with an α-helical content greater than 90 per cent (1,2,3,4,5). In urea or guanidine hydrochloride solutions containing mercaptoethanol or dithiothreitol, the molecule dissociates into two subunits of molecular weight about 35,000 (6,7,8). X-ray diffraction studies provide evidence that the molecule consists of two α-helical chains arranged in an extended coiled-coil configuration. In 8M urea on polyacrylamide or starch gel electrophoresis, the two sub-units appear to migrate as a single band (2,7), a preliminary indication that they are at least very similar if not identical. However, the reduced and alkylated protein is reported to migrate as two components of distinct amino acid composition during gel electrophoresis in the absence of urea (9) while two bands are observed on SDS gel electrophoresis at low protein concentration (10). The N-terminal groups of the protein have been described; glutamic acid or glutamine (11) and an N-acetyl peptide having the

sequence N-Ac-(Met,Asp)-Ala (12). However, Jen et al. (13) were unable to confirm the presence of N-terminal glutamic acid or glutamine. Two C-terminal residues, isoleucine and serine, have been reported (14,15). To provide more definitive chemical evidence on the question of identity or non-identity of the polypeptide chains of tropomyosin, we have undertaken the elucidation of the number of unique sequences about the histidyl, methionyl and cysteinyl residues of this protein.

## MATERIALS AND METHODS

SH-tropomyosin was prepared as described by Bailey (16) in the presence of 0.5 mM dithiothreitol (17) and with an initial two precipitation steps to remove troponin (18). $^{14}$C-labelled CM-tropomyosin* was prepared by treatment of the freeze-dried protein with mercaptoethanol in 8 M urea, precipitation with 33% ethanol-1% conc. HCl-66% acetone (v/v) and alkylation with $^{14}$C-iodoacetic acid. Half-cysteine was estimated from the $^{14}$C incorporation, as carboxymethylcysteine after acid hydrolysis, and as cysteic acid after oxidation of SH-tropomyosin according to Moore (19).

Tryptic, peptic and thermolytic digests of SH-tropomyosin and $^{14}$CM-tropomyosin were prepared in the usual way. The diagonal electrophoretic peptide mapping technique (20,21) at both pH 6.5 and 1.8 was used for the location of cysteic acid and methionine sulfonium salt peptides. Because of poor yields, some methionine peptides were isolated as their sulfones after their location by the diagonal mapping procedure. The $^{14}$C-CMC peptides were separated by Dowex-50 ion-exchange chromatography and high voltage electrophoresis on paper. Amino acid sequences were determined by the 'Dansyl-Edman' technique (22) and in some cases fragments prepared by digestion with trypsin, pepsin or α-lytic protease (23). The assignment of amides was deduced from the electrophoretic mobilities of peptides at pH 6.5 (24).

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* Abbreviation: CM-, carboxymethyl; CM-methionine, S-carbamoylmethylmethionine.

The N-acetyl peptide from a tryptic digest of SH-tropomyosin was isolated both as the CM-methionine and as the methionine sulfone peptide. The former was sequenced by cleavage in water at 100°C (21) and the fragments isolated by electrophoresis at pH 6.5. The sulfone peptide was subjected to partial acid hydrolysis in 0.03 N HCl for 1 hr at 110° and the products separated by pH 6.5 high voltage electrophoresis and paper chromatography using 1-butanol:formic acid:ethyl butyrate:water (400:60:50:100 v/v). The N-acetyl amino acid was detected on the papers with the bromocresol green spray reagent (25) and identified as N-acetyl methionine sulfone by amino acid analysis and comparison with N-acetyl amino acid standards.

## RESULTS AND DISCUSSION

The amino acid composition of the tropomyosin preparation used in this study is in close agreement with those obtained previously by others (Table 1). Of particular interest for the purposes of this work were the histidine and methionine content as well as the levels of cysteine as determined by cysteic acid determinations, incorporation of $^{14}C$-labelled iodoacetic acid into the protein, and the analysis of S-carboxymethyl cysteine after acid hydrolysis. These analyses are all in good agreement with a value of 2.8 - 2.9 residues of cysteine per mole of 70,000. Previous estimates of cysteine content have been variable among different laboratories, possibly arising from troponin contamination in the earliest work and from the difficulty of estimating quantitatively the low level of S-carboxymethylcysteine or cysteic acid in the presence of high levels of several of the other amino acids. To avoid the latter problem, dilute and concentrated aliquots of the hydrolysates were applied to the analyzer columns in the present work. The good agreement obtained among the three methods of estimation lends some confidence to the reliability of these analyses.

The amino acid sequences of the two unique histidyl and five unique methionyl peptides isolated in this work are presented in Table 2. Interestingly,

TABLE 1

AMINO ACID COMPOSITION OF TROPOMYOSIN

(MOLES/70,000 g)

| Amino Acid | Kominz et al.* (1957) | Katz & Converse* (1964) | Carsten* (1968) | Woods* (1969) | This Study |
|---|---|---|---|---|---|
| Lys | 75 | 80 | 74 | 81 | 81[a] |
| His | 3.9 | 4.1 | 3.9 | 4.2 | 4.0[a] |
| Amide $NH_3$ | (45) | - | - | - | - |
| Arg | 29 | 30 | 29 | 30 | 29.5[a] |
| Asp | 62 | 59 | 57 | 64 | 62[a] |
| Thr | 18 | 16 | 15 | 16 | 16.6[b] |
| Ser | 28 | 25 | 26 | 27 | 27[b] |
| Glu | 148 | 150 | 135 | 154 | 151[a] |
| Pro | 1.2 | - | 1.3 | 0-2.9 | 1.0[a] |
| Gly | 9 | 8 | 10 | 9 | 8.0[a] |
| Ala | 77 | 73 | 66 | 79 | 74[a] |
| Cys/2 | 4.6 | - | - | - | - |
| Cys($SO_3$) | - | - | - | - | 2.9[a] |
| CMC | - | - | 1.9 | 2.0-3.2 | 2.8[a,f] |
| Val | 19 | 18 | 17 | 20 | 20.7[c] |
| Met | 11 | 12 | 13 | 14 | 13.2[a] |
| Met$(O)_2$ | - | - | - | - | 13.7[a] |
| Ile | 21 | 22 | 18 | 23 | 23.5[c] |
| Leu | 67 | 66 | 57 | 69 | 67[a,d] |
| Tyr | 11 | 12 | 9 | 11 | 11.5[a] |
| Phe | 2.3 | 2.5 | 3.2 | 2.8 | 3.0[a] |
| Try[e] | 0 | - | - | - | - |

TABLE 1

(Cont'd.)

AMINO ACID COMPOSITION OF TROPOMYOSIN

(MOLES/70,000 g)

* Analyses converted to residues per 70,000 g.

[a] Average of 22- and 70-hour hydrolysates.

[b] Extrapolated to zero time.

[c] Average of 70-hour hydrolysate.

[d] Leucine = 95.0 residues per 100,000 g.

[e] No tryptophan, determined by Bailey (1948), and Kominz et al. (1957).

[f] Value of 2.8 also obtained by radioactive incorporation.

TABLE 2

AMINO ACID SEQUENCES OF HISTIDINE AND METHIONINE PEPTIDES

FROM A TRYPTIC DIGEST OF TROPOMYOSIN

| Peptide | Amino Acid Sequence |
|---|---|
| T-1 | Ala-Ile-Ser-Glx-Glx-Leu-Asp-His-Ala-Leu-Asn-Asp-Met(O)$_2$-Thr-Ser-Ile |
| T-2 | Ala-Ile-Ser-Glx-Glx-Leu-Asp-His-Ala-Leu-Asn-Asp-Met(O)$_2$-Thr |
| T-3 | His-Ile-Ala-Glu-Asp-Ala-Asp-Arg |
| T-4 | Met(O)$_2$-Gln-Met(O)$_2$-Leu-Lys |
| *T-5 | Gly-Met(O)$_2$-Lys |
| *T-6 | N-Ac-Met(O)$_2$-Asp-Ala-Ile-Lys |

* Also isolated as CM-MET peptides.

both N- and C-terminal peptides of tropomyosin were isolated. The sequence of Peptide T6 confirms the presence of the N-acetylated tripeptide previously reported and is consistent with its amino acid composition (12). Peptides T1 and T2, isolated from a tryptic digest of tropomyosin, contain no basic C-terminal residues and are believed to represent two varieties of the C-terminus of tropomyosin preparations. The sequence of T1 is consistent with the previously described carboxypeptidase digests of the intact protein in which isoleucine and serine were liberated (15). However, the presence of a C-terminal threonine residue had not previously been suspected. It seems highly unlikely that T2 could have arisen from T1 from a chymotryptic or other cleavage of the Thr-Ser bond during the tryptic digestion. The presence of at least two chemically different but highly homologous polypeptide chains in tropomyosin preparations is indicated by this finding.

Chemically dissimilar polypeptide chains are also indicated by the isolation and sequence determination of the three unique cysteine peptides shown in Fig. 1. It appears unlikely that any one of these could have arisen from troponin or other impurity in the tropomyosin preparation since the recoveries of A, B, and C were 0.12, 0.30 and 0.14 moles per mole of tropomyosin sub-unit. Significantly, a high degree of homology exists between peptides A and B which differ only in three amino acid residues. These three substitutions are highly conservative in nature. These results are clearly incompatible with a homogeneous tropomyosin preparation of two chemically identical sub-units. Taken together with the histidine and methionine sequences, they are indicative of either non-identical but similar sub-units, or of more than one homologous form of tropomyosin, or both. Obviously, further attempts must be made to isolate chemically pure tropomyosin and its constituent polypeptide chains.

A further point of interest in this work has been the relationship between the postulated coiled-coil structure of tropomyosin and its amino acid sequence. Crick pointed out (26) that the regular interlocking of amino acid side chains

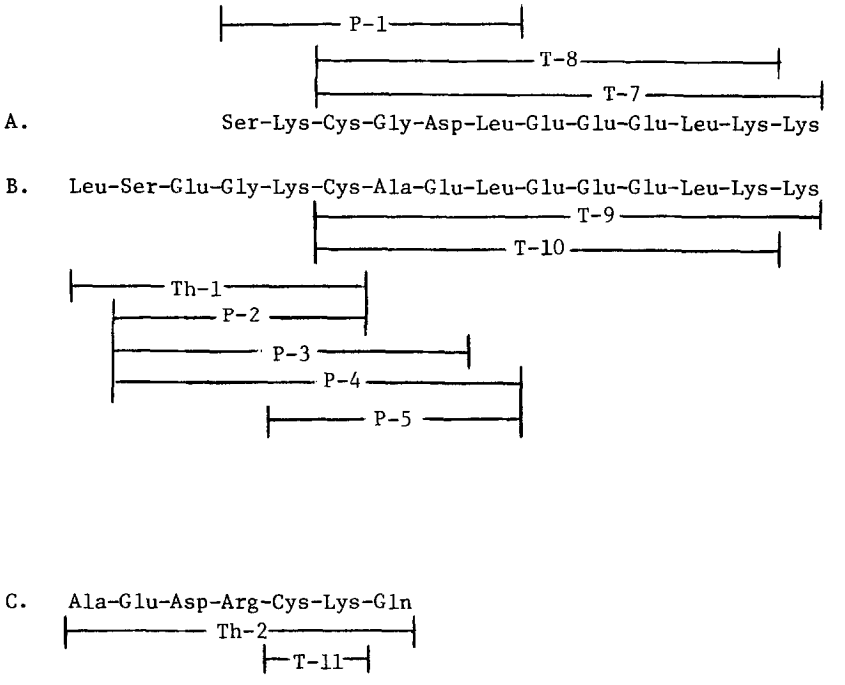


Fig. 1. Unique Amino Acid Sequences of Cysteine Peptides from Tropomyosin. T, Th, and P represent tryptic, thermolytic and peptic peptides. In most cases peptic and tryptic peptides were isolated as both CMC and cysteic acid peptides. Thermolysin peptides were isolated as cysteic acid peptides.

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in a two-stranded coiled-coil structure would predict a regular occurrence of alternating polar and non-polar residues in tropomyosin, the non-polar occurring at an average interval of 3.5 residues. Although the present sequences are not of sufficient length to properly test this prediction, there is a good indication that a periodicity of non-polar residues does occur. Thus in peptides T1 and T2, isoleucine or leucine occur at positions 2, 6, and 10 while in the cysteine peptide B, leucine occurs at positions 1, 9, and 13. The occurrence of lysine at postion 5 of the latter peptide may represent a case in which a charged residue may function as a hydrophobe. This could occur if its side-chain is of sufficient length to permit hydrophobic interaction of its $\beta$ and/or $\gamma$ methylene carbons with other side chains, and at the same time allow its charged moiety to be turned towards the exterior of the coiled-coil. Model

building studies in our laboratory indicate that this may be possible with such residues as lysine, arginine, glutamic acid and others. It will be of great interest to extend the present sequences of tropomyosin to test these tentative observations.

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