Primary Structure of Rabbit Skeletal Muscle Troponin-T

SEQUENCE DETERMINATION OF FOUR CYANOCYNE BROMIDE FRAGMENTS, CB4, CB5, CB6, AND CB7*

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The amino acid sequences of four cyanogen bromide (CB) fragments from rabbit skeletal troponin-T have been determined. Fragment CB4 (55 residues) was documented by chymotryptic, thermolytic, tryptic, and 2-(2-nitrophenylsulfonyl)-3-methyl-3'-bromoidoleinone peptides. A combination of automated sequence analysis, tryptic digestion, and penicilliolecarboxypeptidase-S treatment was used to assemble fragments CB5 (24 residues), CB7 (8 residues), and the COOH-terminal CB6 (21 residues).

Troponin-T is one of three components of the troponin complex (the other two being the calcium-binding protein, tropo- nin-C, and the inhibitory protein, troponin-I) which is intimately associated with tropomyosin to regulate contraction and relaxation in the actomyosin ATPase system. As an initial step in understanding the protein interactions which occur at the molecular level, we have undertaken to establish the amino acid sequence of troponin-T. The first paper of this series (1) was concerned with the isolation and characterization of the four cyanogen bromide fragments. The sequence determination of CB2 was also reported. This paper describes the sequence of four additional fragments, CB4, CB5, CB6, and CB7.

* This work was supported by a Medical Research Council of Canada postdoctoral fellowship to J. R. P. This is the second of a series of three papers which report the sequence determination of troponin-T.

1 The abbreviations used are: Tb-T, troponin-T; BNPS-skatole, 2-(2-nitrophenylsulfonyl)-3-methyl-3'-bromoidoleinone; dansyl, 5-di- methylaminomethyl-1-naphthalene-1-sulfonic acid; PTH, 3-phenyl-2-thiohydantoin; GLC, gas-liquid chromatography; TLCK, N'-p-tosyl-lysine chloromethyl ketone HCl; TLC, thin layer chromatography; DMAA, N,N'-dimethylallylamine.

2 The prefixes used to designate peptides are based on the cleavage methods as outlined in Footnote 2 of the previous paper (1).

3 Some of the data are presented as a miniprint supplement immediately following this paper. Tables I through XI and Fig. 1 are found on pp. 981-982. For the convenience of those who prefer to obtain supplementary material in the form of 18 pages of full size photocopies, they are available as JBC Document Number 76111-914. Orders should specify the title, authors, and reference to this paper, the JBC Document Number, and the number of copies desired. Orders should be directed to The Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Md. 20014, and must be accompanied by a remittance of the order of the Journal in the amount of $2.70 per set of photocopies.

METHODS

Purification of Peptides—The purification and properties of the cyanogen bromide fragments as well as the isolation of smaller peptides from secondary cleavages have been previously described (1).

Proteolytic and Chemical Degradations—N'-p-Tosyl-l-lysine chloromethyl ketone HCl was purchased from Sigma, and BNPS-skatole from Pierce. Penicilliolecarboxypeptidase-S (2) was a gift from Dr. T. Hofmann, University of Toronto. a-Chymotrypsin was treated with TLCK according to the method of Shaw (3). The conditions for digestion with trypsin, a-chymotrypsin, and thermolysin were the same as in the first paper (1). BNPS-skatole was used to cleave at tryptophan by the method of Ommen et al. (4) as modified by Hermanson et al. (5).

Penicilliolecarboxypeptidase-S was dissolved in water at a concentration of 0.54 mg/ml. Peptides (25 to 50 nmol) were dissolved at 37° in 100 μl of 0.03 M pyridine/formate buffer, pH 4.2, and at zero time 15 μl of enzyme was added to the reaction mixture. Aliquots were removed at certain time intervals (given in the text). The reaction was stopped by adding 15 μl of a standard norleucine solution (which had previously been adjusted to pH 2.0 with 12 N HCl) followed by heating at 100° for 5 min.

Peptide Sequencing Procedures—Detailed descriptions of automated sequence analysis, the NH2-terminal dansyl method, amide determination, and manual Edman degradation were previously given (1). A summary of the evidence for acid and amide assignments is given in Table XI. In fragment CB4, when tryptophan was the COOH-terminal residue of a peptide, the dansylated tryptophan was spotted on a thin layer chromatographic plate without prior acid hydrolysis; when tryptophan occupied any other position in a peptide, the dansylated tryptophan was cleaved with TLCK-chymotrypsin in place of 6 N HCl. The Edman procedure was then continued.

Amino Acid Analysis—Samples were hydrolyzed as described in the previous paper (1). Tryptophan was determined by the methanesulfonic acid method of Liu and Chang (6).

RESULTS AND DISCUSSION

Amino Acid Sequence of Fragment CB4—The sequence of fragment CB4 was elucidated using automated sequence analysis (Table I) as well as the subtractive manual procedure on peptides isolated from proteolytic and chemical cleavages of CB4. The properties of peptides derived after digestion of CB4 with TLCK-chymotrypsin, thermolysin, BNPS-skatole, and trypsin are given in Table II. I, III, IV, and V, respectively. Results of penicilliolecarboxypeptidase-S treatment of peptide CB4-T1 are shown in Table VI. Fig. 2 summarizes the peptides used to establish the complete amino acid sequence of CB4. The order of the first 29 residues (176 to 204) was obtained by automated sequence analysis. The remaining C001-terminal portion of CB4 was recovered as a number of chymotryptic...
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Fig. 2. Summary of peptides used to establish the complete amino acid sequence of CB4. →, automated Edman degradation; -, subtractive manual Edman degradation; ---, penicillio carboxypeptidase-S treatment; --, regions inferred from amino acid composition and comparison with analogous peptides.

peptides, each of which was sequenced. The two thermolytic peptides Th8 and Th14 overlapped the regions on either side of tryptophan 205 and tyrosine 227, respectively. BNPS-skatole cleavage at the single tryptophan produced 25-residue peptide B2. This fragment, taken through 14 cycles of the automated Edman procedure, ordered the chymotryptic peptides as being TC5-TC6i-TC6ii and the thermolytic peptides as Th8-Th9-Th10-Th11. The overlap for leucine 222 was provided by the penicillio carboxypeptidase-S results with tryptic fragment T1.

The majority of the acids and amides was determined directly from the mobilities at pH 6.5 of the chymotryptic peptides (Table II). Glutamine 210 was known from peptide Th9 (Table III). Asparagine 188 and aspartic acid 190 were assigned from GLC results (Table I).

CB4, consisting of 55 amino acids, is a basic peptide with a net charge of +4 at neutral pH. Except for 2 clusters of 3 basic residues at the NH2 terminus, the acidic and basic moieties are scattered throughout the entire length of the fragment.

Amino Acid Sequence of CB7 — When 90 nmol of the smallest cyano gen bromide fragment lacking homoserine, it must occupy the COOH-terminal portion of troponin-T. In two separate runs, samples (250 nmol) of CB6 were taken through 20 cycles on the automated sequence analyzer using Beckman program 090872. The results of one such run are shown in Table VII. The sole remaining amino acid was lysine, indicating that it came after tryptophan 258 and was the last residue of CB6 and therefore of troponin-T. Penicillio carboxypeptidase-S results (Table IX) confirmed the sequence at the COOH terminus as being Arg-Trp-Lys. This fragment is devoid of glutamyl and aspartyl residues and has an overall charge of +7 at pH 6.5. The complete sequence of CB6 is shown in Fig. 3.

Amino Acid Sequence of CB5 — Fragment CB5 (185 nmol) was subjected to automated sequence analysis twice using Beckman program 090872. As shown in Table VII, the automated sequenator went through the entire length of CB5, leaving only the COOH-terminal homoserine residue. Alanine at positions 153, 160, 162, and 172 as well as serine at positions 156 and 157 were determined by GLC of the PTH-derivatives. A tryptic digest of CB5 provided peptides (Table VIII) suitable for assigning the acids and amides. Thus asparagine 154, glutamine 170, and glutamic acid 174 were known directly from the mobilities at pH 6.5 of peptides 'T1, 'T5, 'T7, and 'T9. Since residue 163 was found to be aspartic acid from the GLC results of Table VII, the single amide moiety of peptide 'T2 was attributed to glutamine 164.

The COOH terminus of CB5 was found to be Glu-Hse by peptide 'T9 (Table VIII). Further confirmation was obtained by penicillio carboxypeptidase-S treatment of the intact cyano gen bromide fragment (Table X). Although a mixture of fragments CB6 and CB5 was used, the amino acids derived from...
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CB5

\[
\text{Gly-Ala-Asn-Tyr-Ser-Ser-Tyr-Leu-Ala-Lys-Ala Asp Gln Lys-Arg Gly-Lys Lys-Cln-Thr-Ala-Arg-Glu-Hac}
\]

CB7

\[
\text{Asn-Val-Arg-Ala-Arg-Val-Glu-Hse}
\]

CB6

\[
\]

Fig. 3. Summary of evidence used to establish the complete amino acid sequence of fragments CB5, CB7, and CB6. Notations for sequence determination are the same as in Fig. 2.

CB5 could be distinguished from those of CB6 for the following reasons: (a) the sequence of the COOH terminus of CB6 was already known (Table IX); (b) the enzyme cleaved CB6 at a much slower rate than CB5 so that after 3 min tryptophan and lysine were released from CB6 while alanine, arginine, glutamic acid, and homoserine were removed from CB5. The evidence for the complete sequence of CB5 is summarized in Fig. 3.

Acknowledgments — We wish to thank Mr. M. Nattriss and Mrs. Lois Serink for their technical assistance.

REFERENCES

**Sequence of Rabbit Skeletal Troponin-T**

Implement

**The Primary Structure of Rabbit Skeletal Muscle Troponin-T**

by: J. D. Liebman, J. E. Liebman, and G. A. P. D. Smith

**RESEARCH AND DISCUSSION**

**Abstract**

The primary structure of rabbit skeletal muscle troponin-T has been determined. The troponin-T protein from rabbit skeletal muscle was purified and its primary structure was determined by amino acid sequence analysis and mass spectrometry. The sequence of the troponin-T protein is shown in Table 1. The results of this study indicate that the troponin-T protein is composed of three domains: a C-terminal domain, an N-terminal domain, and a hinge domain. The C-terminal domain is responsible for the binding of the troponin-T protein to actin, while the N-terminal domain is responsible for the binding of the troponin-T protein to myosin. The hinge domain is responsible for the flexibility of the troponin-T protein.

**Table 1**

| Peptide | Mass (Da) | Number of Amino Acids | Amino Acid Composition
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>533</td>
<td>52</td>
<td>Ser-Thr-Glu-Ala-Arp</td>
</tr>
<tr>
<td>P2</td>
<td>525</td>
<td>51</td>
<td>Ser-Thr-Glu-Ala-Arp</td>
</tr>
<tr>
<td>P3</td>
<td>515</td>
<td>50</td>
<td>Ser-Thr-Glu-Ala-Arp</td>
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**Summary of Results**

- The troponin-T protein is composed of three domains: a C-terminal domain, an N-terminal domain, and a hinge domain.
- The C-terminal domain is responsible for the binding of the troponin-T protein to actin, while the N-terminal domain is responsible for the binding of the troponin-T protein to myosin.
- The hinge domain is responsible for the flexibility of the troponin-T protein.

**References**


**Supplementary Information**

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Primary structure of rabbit skeletal muscle troponin-T. Sequence determination of four cyanogen bromide fragments, CB4, CB6, and CB7.
J R Pearlstone, M R Carpenter and L B Smillie


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