ISOLATION AND PURIFICATION OF THE CYANOGEN BROMIDE FRAGMENTS FROM TROPONIN I

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1. Introduction

Troponin I is the component of the troponin complex responsible for the inhibition of the Mg^{2+}-activated ATPase of actomyosin in the absence of Ca^{2+} [1]. As a preliminary step towards the determination of its amino acid sequence, troponin I was treated with cyanogen bromide and the fragments so produced were purified by column chromatography and high voltage paper electrophoresis. Ten fragments have been isolated which add up to a total of 180 residues giving a molecular weight for troponin I of 21 000.

2. Materials and methods

Troponin I was isolated from rabbit skeletal muscle as described previously [2].

Cyanogen bromide cleavage was performed in 70% formic acid at room temperature for 24 hr using a 50-fold molar excess of cyanogen bromide over methionine. Excess cyanogen bromide and formic acid were removed by freeze drying after diluting the reaction mixture 10-fold with water. Cysteine residues were alkylated with iodo [^{14}C] acetic acid as described previously [1].

Chromatography of peptides on phosphocellulose (Whatman P11) was performed as described by Chin and Wold [3], the peptides being eluted from the column with a linear gradient of NaCl.

The methods of high voltage paper electrophoresis, amino acid analysis and N-terminal analysis have been described previously [2].

3. Results and discussion

Analysis of troponin I [1, 2] has shown that it contains 8 or 9 residues of methionine per mole and thus cyanogen bromide cleavage, which should result in 9 or 10 peptides, was chosen as a convenient method of producing fragments suitable for sequence analysis.

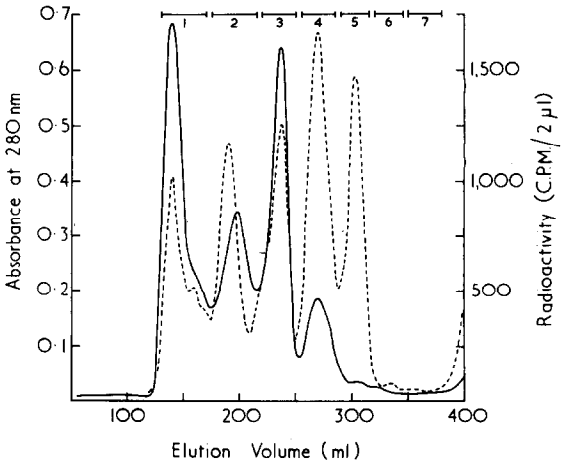

Fig. 1. Gel filtration of a cyanogen bromide digest of troponin I on a column (2.2 × 110 cm) of Sephadex G50 equilibrated with 6 M urea–0.2 M sodium formate, pH 3.5 (——) Absorbance at 280 nm; (– – –) radioactivity. The horizontal bars indicate the fractions which were pooled.

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Troponin I was treated with cyanogen bromide before alkylation in order to avoid any alkylation of the methionine residues, and the alkylation mixture was applied directly to a column of Sephadex G50 equilibrated with a buffer containing 6 M ureau-0.2 M sodium formate, pH 3.5. The result of such a run is shown in fig. 1. Seven fractions were pooled and desalted by passage through a column of Sephadex G10 equilibrated with 10 mM HCl. Samples from each fraction were submitted to amino acid analysis in order to determine the distribution of amino acid residues. Fractions 1 and 2 contained some methionine and would appear to be partial cleavage products and they were not investigated further.

Fractions 3 and 4 were further purified by chromatography on phosphocellulose and the results of these runs are shown in figs. 2 and 3 respectively. Two peaks were pooled from fraction 3, as shown in fig. 2, and these were shown to be single components by electrophoresis. Peak 1 contained all the radioactivity in the fraction and is the fragment CN1, while the UV spectrum of peak 2 showed that it contained the single tryptophan residue of troponin I and is the fragment CN2.

Three peaks were pooled from fraction 4, as shown in fig. 3, and these were again shown to be single components by electrophoresis. Peak 1 contained all the radioactivity in the fraction and is the fragment CN3,

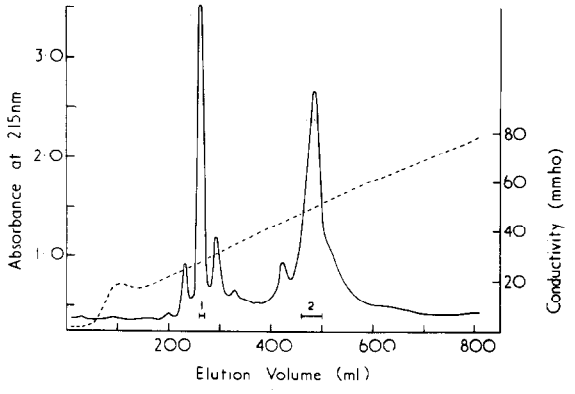

Fig. 2. Chromatography of fraction 3 on a column (1.0 × 22 cm) of phosphocellulose equilibrated with 0.025 N H_3PO_4. The column was eluted with a linear gradient of NaCl from 0 to 1.5 M, the total volume being 800 ml. (——) Absorbance at 215 nm; (– – –) conductivity. The horizontal bars indicate the fractions which were pooled.

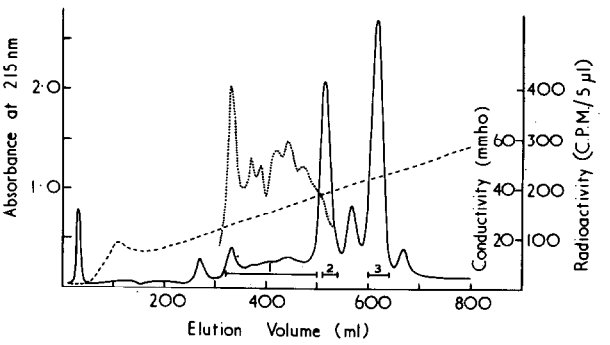

Fig. 3. Chromatography of fraction 4 on a column (1.0 × 22 cm) of phosphocellulose equilibrated with 0.025 N H_3PO_4. The column was eluted with a linear gradient of NaCl from 0 to 1 M, the total volume being 800 ml. (——) Absorbance at 215 nm, (. . . .) radioactivity; (– – – – –) conductivity. The horizontal bars indicate the fractions which were pooled.

while peaks 2 and 3 are the fragments CN4 and CN5 respectively. As can be seen in fig. 3, CN3 was not well resolved on the column and when concentrated it was rather insoluble in water and tended to stick at the origin during electrophoresis, there is nothing particularly abnormal about its amino acid composition and it is difficult to see why it should behave in this way.

The fragments contained in the remaining three fractions, 5, 6, and 7, were isolated by high voltage electrophoresis. Fraction 5 contained fragments CN6 and CN7, fraction 6 contained fragment CN8 and fraction 7 contained fragments CN9 and CN10.

The amino acid compositions of each of the fragments are given in table 1, together with their N-terminal residues.

Together the ten fragments add up to give a total of 181 residues with a molecular weight of 21 000 as compared with the molecular weight of 23 000 estimated by SDS gel electrophoresis [1]. From the yields of each fragment and the compositions of the fractions from the G50 column, it is unlikely that a fragment has been missed, however it will be necessary to obtain overlap information for the fragments, in order to confirm the molecular weight of 21 000. In table 1 the composition derived from the sum of the fragments is compared with that obtained by amino acid analysis [2], and in general there is very good agreement between them.

It has been shown previously that troponin I has a

Table 1
Amino acid composition of the cyanogen bromide fragments of troponin I

	CN1	CN2	CN3	CN4	CN5	CN6	CN7	CN8	CN9	CN10	Total	Troponin I**
					(mole/mole peptide*)							(mole/21 000 g)
Asp	1	6	3	2	2	1	–	–	–	1	16	17.1
Thr	1	1	–	–	1	–	–	–	–	–	3	3.3
Ser	2	1	–	–	1	2	1	–	2	1	10	8.9
Glu	8	6	7	2	3	4	–	1	2	–	33	33.2
Pro	3	–	–	2	–	–	–	–	–	–	5	6.3
Gly	2	2	–	1	1	–	1	1	–	–	8	7.8
Ala	4	1	4	–	2	–	1	–	–	2	14	15.2
Val	–	2	1	1	1	1	1	–	–	–	7	7.1
Ileu	1	1	1	–	1	1	–	–	–	–	5	4.9
Leu	5	2	2	3	2	1	3	–	–	–	18	17.9
Tyr	1	–	1	–	–	–	–	–	–	–	2	2.0
Phe	–	–	–	2	–	–	–	–	1	–	3	2.8
His	1	–	1	–	1	–	1	–	–	–	4	3.6
Lys	2	6	3	3	2	3	3	2	–	–	24	25.8
Arg	2	4	–	5	4	–	–	1	–	–	16	14.3
Trp†	–	1	–	–	–	–	–	–	–	–	1	0.9
Cys†	1	–	1	–	–	–	1	–	–	–	3	2.7
Hsr	1	1	1	1	1	1	1	1	–	1	9	7.4††
Total	35	34	25	22	22	14	13	6	5	5	181	
N-terminus	Leu	Asx	Asx	Ala	–	Glx	Leu	Glx	Phe	Ser		

* Values corrected to nearest integer.
** Recalculated from reference [2].
† Trp and Cys were identified by UV absorption and radioactivity respectively.
†† Estimated as methionine.

blocked N-terminus [2]. CN5 is the only fragment for which no free N-terminal amino acid could be found and CN9 the only one which does not contain homoserine, it seems probably, therefore, that these two fragments respectively form the N- and C-terminal portions of troponin I.

Acknowledgements

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