The Amino Acid Sequence of Troponin I from Rabbit Skeletal Muscle

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The complete amino acid sequence of rabbit skeletal muscle troponin I was determined by the isolation of the cyanogen bromide fragments and the tryptic methionine-containing peptides. Troponin I contains 179 amino acid residues and has a molecular weight of 20864. Its *N*-terminus is acetylated. Detailed evidence on which the sequence is based has been deposited as Supplementary Publication SUP 50055 (23 pages) at the British Library (Lending Division), Boston Spa, Wetherby, West Yorkshire LS23 7QB, U.K., from whom copies may be obtained on the terms given in *Biochem. J.* (1975) 145, 5.

Troponin is a complex of three proteins, troponin I, troponin T and troponin C. This complex, together with tropomyosin, is responsible for the Ca^{2+} -dependent regulation of the actomyosin ATPase (adenosine triphosphatase) in vertebrate striated muscle, and hence for the control of muscular contraction. Troponin I is able, on its own, to inhibit the actomyosin ATPase, but this inhibition is greatly increased by the addition of tropomyosin (Wilkinson *et al.*, 1972). Ca^{2+} sensitivity may be restored to this system by the addition of troponin C, and the interaction of troponin I and troponin C has been well documented (Head & Perry, 1974). In order to obtain a better understanding of the interaction between the various components of the thin filament it is necessary to know the amino acid sequence of each one as a step towards the elucidation of their tertiary structure. So far the sequences of troponin C (Collins *et al.*, 1973), actin (Elzinga *et al.*, 1973) and tropomyosin (Stone *et al.*, 1975) from rabbit muscle have been reported. The present work extends this information to troponin I.

We have previously reported (Wilkinson, 1974a) a method for the purification of troponin I and troponin T and the characterization of these proteins. Troponin I is a basic protein with an isoelectric point of 9.3 and has a blocked *N*-terminal group. Cyanogen bromide cleavage of troponin I gave rise to ten unique fragments (Wilkinson, 1974b), and we have reported the amino acid sequence of two of these fragments (Moir *et al.*, 1974); Huang *et al.* (1974) have also reported similar results. In the present paper we report the amino acid sequence of all the cyanogen bromide fragments and also the isolation of the methionine-containing tryptic peptides which provide overlapping evidence for the fragments. The data have thus been ordered into a single amino acid sequence of 179 residues.

A preliminary account of this work has been given by Wilkinson & Grand (1975).

Materials and methods

Troponin I and its cyanogen bromide fragments were prepared as described previously (Wilkinson, 1974a,b).

After the alkylation of cysteine residues with iodoacetamide, the methionine residues of troponin I were alkylated with iodo[^{14}C]acetic acid by the method of Wilkinson (1968). Labelled protein was dissolved in 1% NH_4HCO_3 and digested with trypsin (2%, w/w); the tryptic peptides were fractionated by chromatography on a column (110 cm $\times$ 1.8 cm) of Sephadex G-25 eluted with 10 mM-HCl, followed by high-voltage electrophoresis.

For sequencing, peptides were further digested with trypsin, chymotrypsin and thermolysin in 1% NH_4HCO_3 and with pepsin in 10 mM-HCl, at an enzyme/substrate ratio of 1:50 (w/w) at 37°C for 3 h.

High-voltage paper electrophoresis, amino acid analysis and dansylation of peptides were carried out as described previously (Wilkinson, 1974b). Peptides containing radioactive cysteine or methionine derivatives were detected, after electrophoresis, by radioautography by using Kodak Blue Brand X-ray films. Specific stains for tyrosine-, tryptophan-, histidine- and arginine-containing peptides were used as described by Easley (1965). The dansyl-Edman procedure was carried out as described by Gray (1967) and hydrazinolysis by the method of Bradbury (1956).

Amide groups were assigned to aspartyl and glutamyl residues on the basis of the electrophoretic mobility of the peptides concerned at pH 6.5 as described by Offord (1966). All mobilities were measured relative to aspartic acid.

Results and discussion

The strategy adopted for the sequencing of troponin I was to isolate and sequence the fragments

Fig. 1. Amino acid sequence of troponin I

The order of the cyanogen bromide fragments is as shown. The overlapping tryptic peptides are indicated by solid lines.

produced by cyanogen bromide cleavage and then to place these in their correct order by isolating the methionine-containing tryptic peptides after radioactive labelling of the methionine residues. The sequence is presented here in Fig. 1, and the detailed evidence on which it is based has been deposited as Supplementary Publication SUP 50055 at the British Library (Lending Division). The deposited data contain details of the composition and electrophoretic mobility of the peptides obtained by enzymic digestion of the cyanogen bromide fragments and also the Edman degradations performed on these peptides. It also contains similar details of the methionine-containing peptides isolated from a tryptic digest of the whole protein.

As previously reported (Wilkinson, 1974b) ten unique cyanogen bromide fragments were isolated, which together account for the amino acid composition of troponin I. The larger fragments were subdigested with trypsin, chymotrypsin and thermolysin and the peptides obtained were purified by high-voltage electrophoresis and sequenced by the dansyl-Edman procedure. The two smallest fragments, CN9 and CN10, were sequenced directly.

No particular problems were encountered with the sequence except for the *N*-terminal peptide, CN5-T1-P1, which has the sequence AcGly-Asp-Glu. It was prepared by digestion of either peptide CN5 or CN5-T1, the *N*-terminal tryptic peptide of CN5, with pepsin for 16h followed by passage of the digest through a small (0.6cm×9cm) column of Zeo-Karb 225 (H⁺ form), CN5-T1-P1 being the only peptide unretarded by the column. The sequence of the peptide and confirmation of the acetyl blocking group were obtained by mass spectrometry, which was kindly performed by Dr. Howard R. Morris. For peptides containing histidine and carboxymethylcysteine it was necessary to use a subtractive Edman technique when these residues became *N*-terminal.

The overlapping peptides for the cyanogen bromide fragments were obtained by tryptic digestion of troponin I in which the methionine residues had been alkylated with iodo[¹⁴C₂]acetic acid. Eight major and several minor peptides were obtained and the *N*-terminal sequence of each peptide was determined. This information, together with the known sequence of the *N*- and *C*-terminal tryptic peptides from each of the cyanogen bromide fragments, allows the sequence to be aligned as shown in Fig. 1. All the peptides obtained were derived from expected tryptic cleavage, although in some cases partial cleavage of some trypsin-sensitive bonds gave rise to minor peptides; this was so with the methionine residues at positions 21, 81, 135 and 174. In agreement with the observation of Platt *et al.* (1973), no dansyl derivative was detected when carboxymethylmethionine was *N*-terminal in peptides, but there was no difficulty in determining the subsequent residue.

The only peptide to give difficulty was TA1, which should have glutamine *N*-terminal, and it seems likely that this cyclized to pyrrolidonecarboxylic acid during isolation; indeed the corresponding peptide from peptide CN1 was isolated with both a free and a blocked *N*-terminus. Peptide TB4b gave twice the amount of methionine after hydrolysis and had approximately twice the specific radioactivity of the other peptides, and thus contained two methionine residues spanning positions 117 and 122.

All of the radioactive peptides isolated could be accounted for by the sequences around the nine methionine residues of troponin I, allowing the ordering of the ten cyanogen bromide fragments into a single sequence of 179 amino acid residues with a molecular weight of 20864. This is somewhat lower than the commonly accepted value of 23000 daltons as determined by sodium dodecyl sulphate-polyacrylamide-gel electrophoresis (Wilkinson *et al.*, 1972).

We have shown (Moir *et al.*, 1974) that two positions in troponin I, residues 11 and 118, can be phosphorylated, and Huang *et al.* (1974) have reported two further sites of phosphorylation at residues 19 and 89. Huang *et al.* (1974) have reported the amino acid sequences around the phosphorylated residues, and their results agree with those presented here, except for the sequence Val-Gln at position 85/86, which is inverted in their data.

Troponin I has 13 amide groups, 35 acidic side chains and 44 basic side chains, if histidine is included, and this charge distribution is in agreement with the value of 9.3 previously determined for the isoelectric point (Wilkinson, 1974a).

No sequence homology is apparent with any of the thin-filament proteins referred to above or with the sequence of the myosin alkali light chain obtained by Frank & Weeds (1974). The most interesting feature of the sequence is the highly basic region from residue 102 to 135, which contains 12 basic and only one acidic side chains in a stretch of 34 residues; this region contains the serine residue at position 118 which can be phosphorylated with the cyclic AMP-dependent protein kinase. There is also a lesser basic region at the *N*-terminal end from residue 5 to 27, which includes the threonine residue at position 11 which is phosphorylated by phosphorylase *b* kinase.

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