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Affinity-Chromatographic Isolation and Some Properties of Troponin C from Different Muscle Types

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1. The formation of a complex between troponin I and troponin C that is stable in 6M-urea and dependent on Ca^{2+} was demonstrated in extracts of vertebrate striated and smooth muscles. 2. A method using troponin I coupled to Sepharose is described for the rapid isolation of troponin C from striated and smooth muscles of vertebrates. 3. Troponin C of rabbit cardiac muscle differs significantly in amino acid composition from troponin C of skeletal muscle. The primary structures of troponin C of red and white skeletal muscle are very similar. 4. The troponin C-like protein isolated from rabbit uterus muscle has a slightly different amino acid composition, but possesses many similar properties to the forms of troponin C isolated from other muscle types. 5. The electrophoretic mobilities of the troponin I–troponin C complexes formed from components isolated from different muscle types are determined by the troponin I component.

It is now well established that the composition of the troponin complex is not identical in different muscle types (see Perry, 1974, for review). Thus it appears that, as with myosin and with tropomyosin (Cummins & Perry, 1973, 1974), the structures of the components of the troponin complex vary with muscle speed and activity pattern. Knowledge of the structural changes in the components of the troponin complex that occur in response to the activity pattern of different muscle types should throw light on the structure and function relationships of these proteins and on the possible relationships between their synthesis and the nervous regulation of different muscle types. Practically all the reports of differences in the nature of the components have been concerned with the troponin complex isolated from white skeletal and cardiac muscle (Greaser *et al.*, 1972; Tsukui & Ebashi, 1973; Dabrowska *et al.*, 1973; Head & Perry, 1974; Syska *et al.*, 1974; Hirabayashi & Perry, 1974; Brekke & Greaser, 1976).

Direct isolation of troponin I from whole muscle by the use of affinity chromatography exploiting the strong complex formed between troponin I and troponin C in the presence of Ca^{2+} (Perry *et al.*, 1972; Head & Perry, 1974), has shown that white skeletal, red skeletal and cardiac muscles of the rabbit contain specific forms of troponin I differing from each other in amino acid analysis, molecular weight and biological activity (Syska *et al.*, 1974). It has also been established that troponin T exists, in the red and white muscles of the chicken (Hitchcock, 1973; Perry & Cole, 1974), in forms that migrate differently on electrophoresis in sodium dodecyl sulphate. In addition there is evidence that the form of troponin T in white skeletal muscle differs from that present in cardiac muscle (Greaser *et al.*, 1972; Cole & Perry, 1975; Brekke & Greaser, 1976).

Thus for troponin I and troponin T there are differences of primary structure and molecular weight in the proteins present in white compared with those in red skeletal muscle and in cardiac muscle. Unlike the other components of the troponin complex, troponin C does not appear to change in molecular weight with muscle type (Hirabayashi & Perry, 1974; Head & Perry, 1974). Although troponin C from white skeletal muscle differs in a number of respects from that present in cardiac muscle of the same species (Hirabayashi & Perry, 1974) there is no information as to whether the structure of the protein changes with speed of contraction in skeletal muscle.

The current methods used for the isolation of troponin C are based on the isolation of the troponin complex followed by fractionation into its components. They tend to be time-consuming, require relatively large samples of muscle and frequently difficulties are experienced in applying the standard methods for troponin preparation to muscle types other than skeletal. To facilitate the comparative study of troponin C, an affinity-chromatographic procedure involving troponin I linked to Sepharose has been developed from that described for the isolation of troponin I from

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vertebrate muscle (Syska *et al.*, 1974). The method has been used for the direct isolation of troponin C from whole muscle, enabling a rapid study of the distribution and nature of the protein from relatively small samples of different muscle types. Troponin C of cardiac muscle differs from that of smooth muscle and from troponin C of white skeletal muscle in the rabbit. Troponin C from white skeletal muscle appears to be very similar to, if not identical with, the troponin C of red skeletal muscle.

Methods

Troponin and its components

The troponin complex was prepared by the method of Ebashi *et al.* (1971), with the modification that one rather than two washes with 0.4M-LiCl was used. Troponin I and troponin C were isolated from the complex by the method described (Perry & Cole, 1974; Head & Perry, 1974). In some cases troponin I was isolated directly from whole muscle by affinity chromatography (Syska *et al.*, 1974).

Electrophoresis

This was carried out in the presence of 6M-urea as described by Head & Perry (1974), by using 8% (w/v) polyacrylamide gels for whole muscle extracts and troponin components and 15% polyacrylamide gels for CNBr digests of troponin C.

Samples (about 1g) of whole muscle were prepared for electrophoresis by blending the freshly excised intact muscle in about 2vol. of 8M-urea/20mM-Tris/HCl (adjusted to pH7.8 with 100mM-HCl)/50mM-2-mercaptoethanol, in a small Waring-blender-type homogenizer. The suspension was then centrifuged at 70000*g* for 30min to remove particulate material and the supernatant was used for electrophoresis. For electrophoresis in the absence or presence of calcium, 5mM-EGTA [ethanedioxybis-(ethylamine)tetra-acetate] or 1mM-$CaCl_2$ respectively was included in the sample applied to the gel.

Electrophoresis in 0.1% sodium dodecyl sulphate was carried out on 10% polyacrylamide gel by the method of Weber & Osborn (1969), in 100mM-sodium phosphate buffer, pH7.0.

Preparation of affinity columns

CNBr activation of Sepharose 4B was carried out essentially by the methods of Cuatrecasas *et al.* (1968), by using 50mg of CNBr/g of packed Sepharose, and of March *et al.* (1974), by using 400mg of CNBr/g of packed Sepharose. Troponin I from rabbit white skeletal muscle and troponin I–troponin C mixtures (both proteins isolated from rabbit white skeletal muscle) were coupled to the Sepharose (2mg of troponin I/g of packed Sepharose) in the presence of 0.2M-$NaHCO_3$ at pH9.5 and 4°C. Troponin I (10mg/ml) was dialysed exhaustively against 2mM-HCl before coupling, and troponin I–troponin C mixtures (5mg of troponin I/ml and 5mg of troponin C/ml) were dialysed against 1mM-$CaCl_2$/0.2M-$NaHCO_3$, pH9.5 at 4°C. Coupling was carried out at 4°C with continuous gentle mixing for 12h. After coupling the gel was suspended in 1M-ethanolamine adjusted to pH8.0 with 1M-HCl for 2h at 22°C to block any remaining activated groups on the Sepharose.

After coupling, the protein–Sepharose matrices were washed at 22°C with 8M-urea/20mM-Tris/10mM-EGTA/15mM-2-mercaptoethanol adjusted to pH7.8 with 1M-HCl (500ml for 10g columns), to remove unbound protein. The washings were retained and an estimate of the protein bound to the Sepharose was made from the A_{280} of the combined washings. Specific extinction coefficient at 280nm for troponin I = 3.97 (Wilkinson, 1974). Before use stock solutions of the urea were passed through a column of Zerolit DM-F ion-exchange resin (BDH Chemicals, Poole, Dorset, U.K.) to remove any contaminating cyanate and other ions.

With 20mg of troponin I and 10g of activated Sepharose, more than 95% of the applied protein was bound to the Sepharose; with similar amounts of troponin I–troponin C mixtures about 75% of the total applied protein was bound.

Use of affinity columns

Samples for application to affinity columns were prepared by dispersing 2–30g of freshly excised whole muscle in 4vol. of 8M-urea/20mM-Tris/HCl (pH7.8)/15mM-2-mercaptoethanol/1mM-$CaCl_2$ in a small Waring-blender-type homogenizer. Muscle extracts were clarified by centrifugation at 70000*g* for 30min, dialysed at 22°C for at least 4h against the urea buffer used for homogenization and applied to columns (1.5cm × 10cm) of the Sepharose–protein matrices previously equilibrated with the buffer in which the muscle was homogenized. Unbound protein was eluted from the column by washing with 500ml of 8M-urea/1mM-$CaCl_2$/20mM-Tris/HCl (pH7.8)/15mM-2-mercaptoethanol. Bound protein was eluted with 8M-urea/10mM-EGTA/20mM-Tris/HCl (pH7.8)/15mM-2-mercaptoethanol. Chromatography was carried out at 22°C.

Owing to the low molar extinction coefficient of troponin C the elution of small quantities of this protein from the affinity column could not be followed by monitoring the A_{280} of the eluate. In these cases the protein peak was detected by carrying out electrophoresis in 6M-urea, pH8.6, on 100μl portions of each 5ml fraction eluted after application

of the EGTA step to the column. The point at which the protein started to be eluted was detected by the sharp increase in conductivity of the eluate when the EGTA front emerged from the column. In the standard procedure using 10g of muscle and a troponin I–Sepharose column (1.5cm×10cm), all the troponin C bound to the column was eluted in about 20ml after the EGTA front. The fractions containing protein were pooled, dialysed against water and freeze-dried. Troponin C isolated by this procedure migrated as a single band when up to 100μg was examined by electrophoresis in sodium dodecyl sulphate, pH7.0, or 6M-urea, pH8.6.

Protein determinations

Protein was measured by total N estimation by the Nesslerization procedure described by Strauch (1965) by assuming that protein samples contained 16% N, or by the method of Folin (Lowry *et al.*, 1951), with bovine serum albumin as a standard.

CNBr cleavage

This was carried out by dissolving the protein in 70% (v/v) formic acid (Steers *et al.*, 1965) to a concentration of 5–10mg/ml, and adding 25mg of CNBr/ml of sample. The reaction was allowed to continue for 20h at room temperature, after which time it was diluted with 20vol. of water and freeze-dried.

Results

Troponin C–troponin I complex formation in different muscle types

Experience with the use of troponin C–Sepharose columns for the direct isolation of troponin I from muscle suggested that, provided that troponin I could be linked to Sepharose without significant loss of its ability to form a specific complex with troponin C, the affinity-chromatographic method could be adapted for direct isolation of troponin C from whole vertebrate striated muscle. To extend the method to other muscle types it was also necessary to establish whether a troponin I–troponin C complex stable at high urea concentration was formed with preparations from vertebrate smooth and invertebrate striated and smooth muscles. This was carried out by comparing the electrophoretic patterns of whole muscle extracts in 6M-urea/25mM-Tris/80mM-glycine buffer, pH8.6, in the presence or absence of Ca^{2+} (see the Methods section). In the presence of Ca^{2+} the troponin I–troponin C complex migrated with an intermediate electrophoretic mobility together with the other protein bands and often could not be resolved from them. On removal of free Ca^{2+} by the addition of EGTA the complex was dissociated; the troponin I stayed at or close to the origin at this pH value, but the troponin C was apparent as a single band migrating to the anode more rapidly than any other protein. All smooth and striated rabbit muscles analysed in this way showed the presence of a Ca^{2+}-dependent complex. Likewise skeletal muscles from cow, rat, guinea pig, chicken, cod, carp, bream and smooth muscle of the bovine aorta, rabbit uterus and chicken gizzard also gave evidence of the presence of such a complex when they were examined by this technique (Plate 1).

When similar experiments were carried out on muscle from invertebrates no such complex-formation was observed. It is possible that a troponin I–troponin C complex is dissociated by EGTA, but that the troponin C band produced is masked by other bands. The muscles studied included the whole adductor of *Pecten maximus*, the flight and leg muscles of the locust (*Schistocerca gregaria*) and samples from the giant barnacle (*Balanus nubilis*), octopus (*Octopus macropus*) and crab (*Carcinus maenas*).

Affinity chromatography of troponin C on troponin I–Sepharose columns

Coupling of troponin I to Sepharose. Columns of troponin I–Sepharose made by the procedures of Cuatrecasas *et al.* (1968) and March *et al.* (1974) both bound troponin C selectively when extracts of psoas muscle in 8M-urea/20mM-Tris/HCl (pH7.8)/1mM-$CaCl_2$ were applied (see the Methods section). It can be seen from Fig. 1 that with a 10g troponin I–Sepharose column prepared by the procedure of March *et al.* (1974), the amount of troponin C isolated increased steadily when increasing amounts of muscle were applied. On average about 1.5mg of troponin C was isolated from 5g of fresh muscle, and the yield did not increase when larger amounts of muscle were applied, indicating that the column was saturated. This weight of muscle contains about 6mg of troponin C (Head & Perry, 1974). Under similar conditions, with troponin I–Sepharose prepared by the method of Cuatrecasas *et al.* (1968), the yields of troponin C were significantly lower, on average about 600μg from 10g of muscle. In both cases 100μg samples of the troponin C eluted by application of buffer containing EGTA migrated as a single band corresponding to a molecular weight of about 18000 on electrophoresis in sodium dodecyl sulphate. On electrophoresis in 6M-urea, pH8.6, the troponin C migrated as a single band (Plate 2), and in the presence of Ca^{2+} formed a complex of lower mobility on addition of troponin I from rabbit white skeletal muscle. This complex was dissociated when 5mM-EGTA was added to the sample before electrophoresis (Head & Perry, 1974).

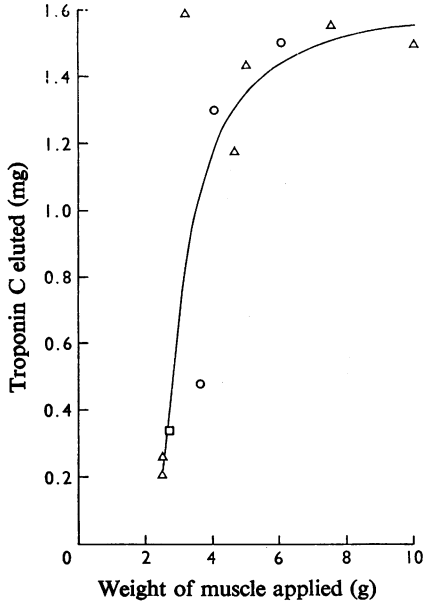


Fig. 1. *Relation between yield of troponin C and amount of rabbit muscle applied to an affinity column prepared by coupling troponin I to Sepharose*

Muscle extracts were applied as described in the Methods section. Columns (10g) were prepared by the method of March *et al.* (1974), and protein was determined by method of Lowry *et al.* (1951). △, Psoas; □, crureus; ○, soleus.

If an extract of cardiac muscle was applied to troponin I–Sepharose columns prepared by the method of Cuatrecasas *et al.* (1968), no troponin C could be eluted by EGTA buffer, and only small variable amounts of material that was polydisperse on electrophoresis in sodium dodecyl sulphate were eluted from columns prepared by the procedure of March *et al.* (1974). On subsequent addition of a skeletal-muscle extract to a column that had failed to bind troponin C from cardiac-muscle extracts, skeletal troponin C was bound in the usual amounts. Extracts of red rabbit muscle, such as the soleus and crureus, behaved similarly to those made from psoas muscle in that the columns prepared from troponin I–Sepharose invariably gave similar yields of troponin C (Fig. 1).

Troponin I–troponin C complex coupled to Sepharose. It was considered that the ineffectiveness of the troponin I columns for the isolation of cardiac troponin C was due to some modification of the troponin C-combining site of troponin I during the coupling to Sepharose. Binding of troponin C probably occurs at the *N*-terminal region of troponin I and may also involve the peptide region represented by residues 96–117 (Perry *et al.*, 1975; Syska *et al.*, 1976). Both of these regions contain several lysine residues that may be involved in forming covalent links to the Sepharose. In an attempt to protect the lysine groups in the troponin C-binding region, a troponin I–troponin C complex containing the protein in equimolar amounts was coupled to Sepharose by the methods of Cuatrecasas *et al.* (1968) and March *et al.* (1974). This procedure has the added advantage that the complex was soluble in the pH range in which the coupling reaction was carried out. When troponin I alone was coupled to Sepharose, it was dissolved initially in 2mM-HCl and partially came out of solution when the pH was subsequently adjusted to pH9.0.

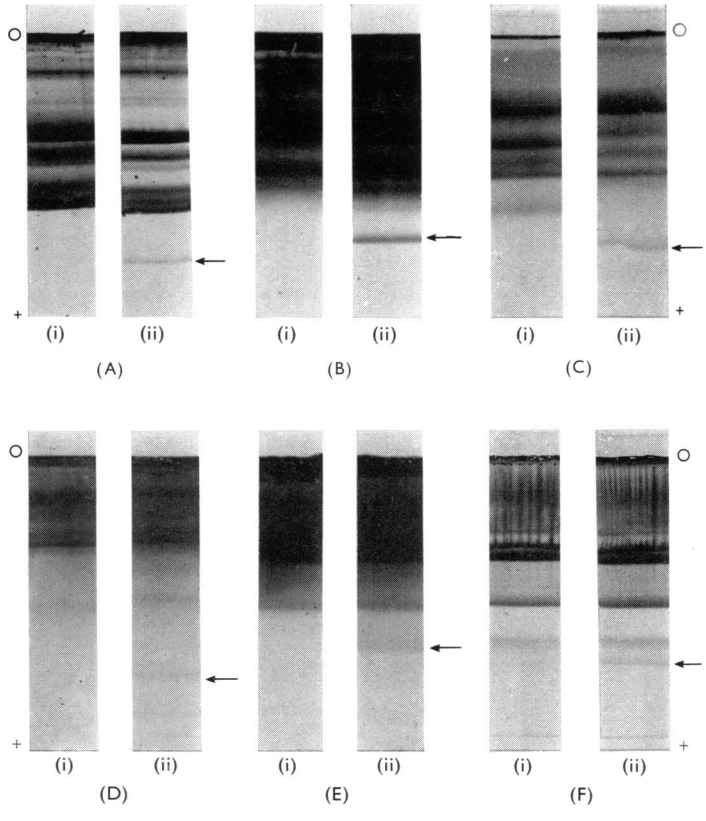
After coupling the troponin I–troponin C complex little if any troponin C capable of binding troponin I was bound to the Sepharose if the column was well washed in 8M-urea/20mM-Tris/HCl (pH7.8)/15mM-2-mercaptoethanol/10mM-EGTA before use. This was evident from the fact that only troponin C could be eluted from such columns by the urea buffer system containing 10mM-EGTA after whole muscle extracts in the urea buffer containing $CaCl_2$ had been applied to them. Further, after troponin I alone, dissolved in the urea buffer containing 1mM-$CaCl_2$, was applied and the column washed with the same buffer, no significant amounts of protein were eluted when washing was continued with urea buffer in which the $CaCl_2$ was replaced by 10mM-EGTA.

With the troponin I–Sepharose column prepared from the complex, satisfactory yields of troponin C were obtained from whole psoas and cardiac-muscle extracts. As with affinity columns prepared from troponin I, those prepared from the complex by the method of March *et al.* (1974) gave better yields of troponin C from all muscle types. The method of March *et al.* (1974) was therefore adopted as the method of choice for coupling the proteins to Sepharose. Somewhat surprisingly, the yields of troponin C from cardiac muscle were better than those obtained from red or white skeletal muscle (Fig. 2). Columns of 10g wet wt. were saturated with troponin C when about 5g of cardiac muscle was applied; under these conditions about 2mg of troponin C could be isolated from the muscle.

After continued re-use, the yield and purity of the troponin C isolated from whole muscle extracts by troponin I–Sepharose columns, whether they were prepared from troponin I or the troponin I–troponin C complex, deteriorated. Columns were normally not used for more than six cycles of application and elution.

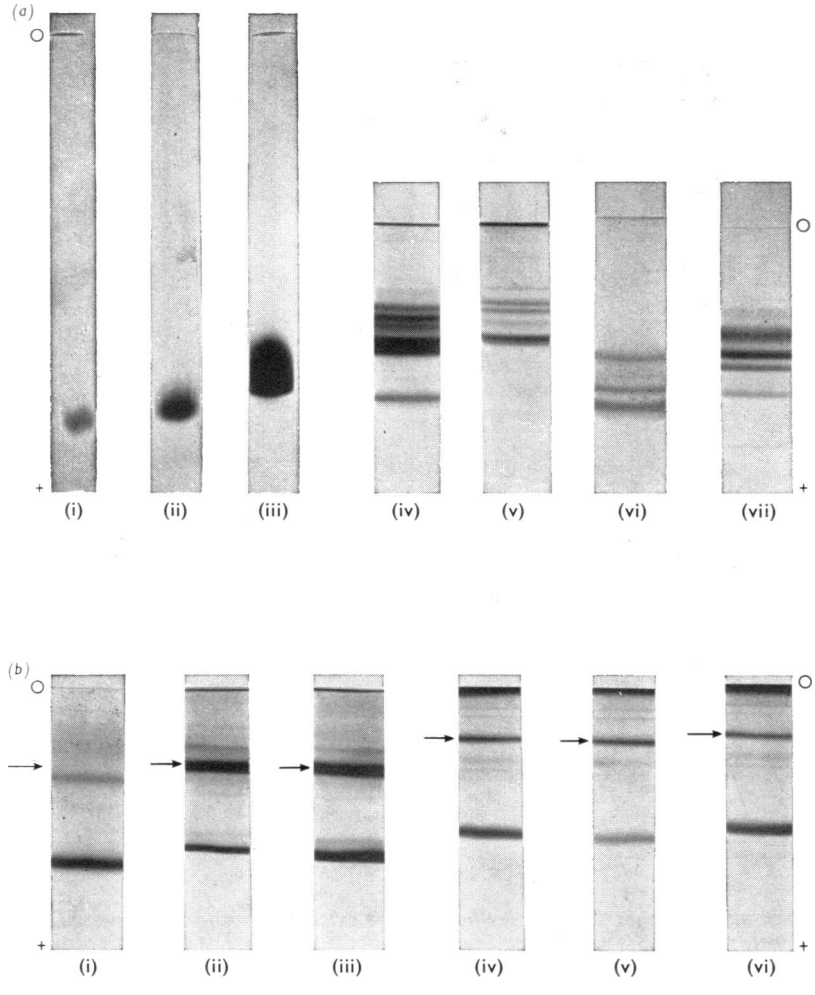
Properties of troponin C from different muscle types

Striated muscle. In general the amino acid analysis of troponin C from rabbit cardiac muscle was



EXPLANATION OF PLATE 1

Evidence for the involvement of troponin C in complex-formation in extracts of whole vertebrate striated and smooth muscle
Extracts of muscle were made in 8M-urea/20mM-Tris/HCl buffer (pH7.8)/50mM-2-mercaptoethanol as described in the Methods section and 50μl samples were applied to polyacrylamide gel and electrophoresis was carried out in 6M-urea/25mM-Tris/80mM-glycine, pH8.6, with additions to the samples as indicated. Presumed troponin C is indicated by an arrow. O, Origin; gel (A) cod white skeletal; gel (B) rat skeletal; gel (C) carp white skeletal; gel (D) rabbit uterus; gel (E) ox aorta; gel (F) chicken gizzard. In each case (i) indicates with 1mM-$CaCl_2$ and (ii) indicates with 5mM-EGTA.



EXPLANATION OF PLATE 2

Electrophoresis of troponin C isolated from different rabbit muscles

(*a*) Purity of protein samples and composition of CNBr digests. Whole protein applied in (i) heart (50μg), (ii) soleus (50μg), (iii) uterus (30μg); CNBr digests (100μg) applied in (iv) longissimus dorsi (v) soleus (vi) uterus (vii) heart. Differences in staining intensities of troponin C samples were not significant. (*b*) Complexes formed between different polymorphic forms of troponin I (50μg) and troponin C (100μg) from rabbit muscles. Band of troponin I–troponin C complex is indicated by the arrow. (i) Fast white troponin I and fast white troponin C, (ii) fast white troponin I and cardiac troponin C, (iii) fast white troponin I and soleus troponin C, (iv) cardiac troponin I and cardiac troponin C, (v) cardiac troponin I and longissimus dorsi troponin C, (vi) cardiac troponin I and soleus troponin C.

similar to that of troponin C isolated from white skeletal muscle (Table 1). There were, however, relatively small but distinct differences, particularly in the alanine, valine, isoleucine, leucine, methionine, phenylalanine, lysine and possibly arginine contents. The differences were very similar to those reported previously (Hirabayashi & Perry, 1974) between troponin C from cardiac and skeletal muscle of the chicken. Indeed the amino acid compositions of cardiac troponin C from chicken and rabbit were more alike than were those of the skeletal and cardiac troponin C from the same species. van Eerd & Takahashi (1975) have also reported considerable differences in amino acid sequence and composition between the troponin C of white skeletal muscle of the rabbit and that of bovine heart. In the rabbit both forms of troponin C contained tyrosine, whereas this residue is absent from chicken skeletal-muscle troponin C (Hirabayashi & Perry, 1974).

The electrophoretic patterns, obtained on 15% polyacrylamide gel in 25mM-Tris/80mM-glycine buffer, pH8.6, of the mixture of peptides obtained by treatment of troponin C from cardiac and white skeletal muscles with CNBr exhibited marked differences. CNBr digests of troponin C isolated from the soleus or crureus muscles of the rabbit gave very similar electrophoretic patterns to those obtained with troponin C isolated from the psoas and longissimus dorsi muscles of the same species (Plate 2*a*).

At pH8.6, in the presence of Ca^{2+}, the troponin C isolated from rabbit cardiac and soleus muscles formed complexes with troponin I from white skeletal muscle. These complexes were stable in 6–8M-urea and had similar electrophoretic mobilities to those of the complex obtained when both troponin components were isolated from rabbit white skeletal muscle (Table 2). Likewise

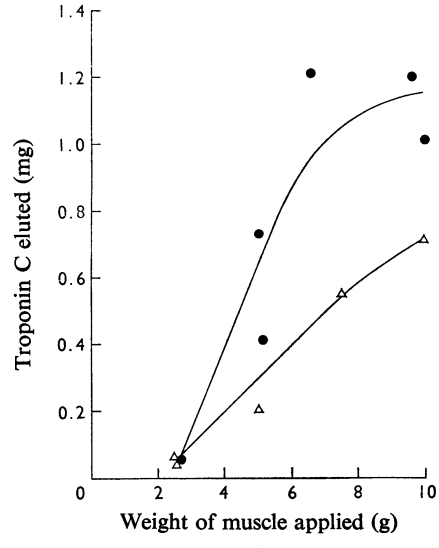


Fig. 2. *Relation between yield of troponin C and amount of rabbit muscle applied to an affinity column prepared by coupling troponin I–troponin C complex to Sepharose*
Conditions were as for Fig. 1. △, Psoas; ●, heart.

Table 1. *Amino acid composition of troponin C from different muscle types*
Analyses were carried out in duplicate after 24 and 72h of hydrolysis as described by Wilkinson *et al.* (1972).

Source ...	Chicken heart*	Rabbit heart	Rabbit uterus	Rabbit soleus	Rabbit white skeletal†
Asp	24.6	25.2	22.8	21.9	22
Thr	7.6	6.6	12.3	8.0	6
Ser	4.9	6.0	10.2	8.5	7
Glu	30.2	29.4	29.6	30.7	31
Pro	3.5	1.0	3.0	3.0	1
Gly	12.9	12.7	16.6	12.1	13
Ala	7.8	9.9	13.1	12.1	13
Val	7.9	8.2	6.9	7.0	6
Met	9.3	8.3	8.4	8.4	10
Ile	8.1	8.7	6.6	8.5	10
Leu	12.5	13.6	9.2	9.4	9
Tyr	2.9	2.7	2.2	2.1	2
Phe	8.3	8.0	7.5	9.0	10
His	0.7	1.2	1.7	1.4	1
Lys	13.1	13.3	10.5	8.8	9
Arg	4.2	5.4	5.4	8.2	7
No. of preparations		4	3	1	

* Hirabayashi & Perry (1974).
† From the primary sequence (Collins, 1974).

Table 2. *Electrophoretic mobilities of complexes formed between troponin I and troponin C from different muscles of the rabbit*

Values are given ±S.D. for four experiments. Mobilities are expressed as the ratio of the rates of migration of the complex to that of the troponin C used to form the complex.

Source of troponin I	Source of troponin C: Longissimus dorsi	Heart	Soleus
Longissimus dorsi	0.50±0.007	0.49±0.007	0.49±0.006
Heart	0.36±0.009	0.35±0.016	0.35±0.015

troponin I from rabbit heart formed complexes with the troponin C from the red skeletal, white skeletal and cardiac muscles with very similar mobilities that were lower than those obtained with troponin I skeletal muscle (Plate 2). Thus the electrophoretic mobilities of the complexes under these conditions were determined by the polymorphic form of the troponin I rather than that of the troponin C (Table 2).

Smooth muscle. The band patterns on electrophoresis in 6M-urea, pH7.8, obtained with extracts of whole smooth muscle in the presence and absence of Ca^{2+} (Plate 1) indicated that a troponin C-like component and a component presumably similar to troponin I of skeletal muscle, which gave rise to a complex in the presence of Ca^{2+}, were also present in this tissue. When whole rabbit uterus extracts in 8M-urea/20mM-Tris/HCl buffer (pH7.8)/15mM-2-mercaptoethanol were applied to a troponin I–Sepharose column, a troponin C-like component could be extracted from the tissue. The yields were low and amounted to about 200μg per 5g wet wt. of muscle applied to a 10g troponin I–Sepharose column. The material moved as a single band corresponding to an apparent molecular weight of about 18000 on electrophoresis in sodium dodecyl sulphate, pH7.0.

The troponin C-like protein from rabbit uterus clearly interacted with white-skeletal-muscle troponin I, for this property was used in its isolation. Its amino acid content, although showing similarities to that of troponin C from white skeletal muscle, had significant differences, particularly in the threonine, serine, proline, glycine and isoleucine contents (Table 1). The appearance of the bands obtained on electrophoresis of CNBr digests was not identical with those obtained with similar digests of troponin C isolated from rabbit white and red skeletal muscles nor from cardiac muscle (Plate 2*a*).

Discussion

The formation of the Ca^{2+}-dependent troponin I–troponin C complex which is stable in 6–8M-urea and can be demonstrated by electrophoresis of whole muscle extracts, provides a convenient method for the detection of troponin I and troponin C, and presumably the whole troponin complex in muscle extracts. This screening procedure indicates that a troponin I–troponin C-like complex that is Ca^{2+}-dependent is also present in smooth muscle from rabbit uterus, chicken gizzard and cow carotid.

The fact that a troponin I–troponin C-like complex could not be demonstrated with extracts from invertebrate muscle does not necessarily indicate the absence of the troponin system from some of these muscles at least, but more likely that the complex in the invertebrate muscles tested is not stable in the 6M-urea system used for electrophoresis.

It is clear from the results obtained with the troponin C–Sepharose affinity column reported elsewhere (Syska *et al.*, 1974), and those obtained with troponin I–Sepharose column described in the present paper, that the troponin I–troponin C interaction is specific for neither species nor muscle type. There appear, however, to be differences in the ability of troponin I linked to Sepharose to bind troponin C in extracts of different types of muscle. The observation that skeletal troponin I linked to Sepharose, without protection of the troponin C-binding site, would bind skeletal (but not cardiac) troponin C suggests that differences exist in the affinity of white-skeletal-muscle troponin I for the skeletal and cardiac forms of troponin C. This is not perhaps surprising, for these proteins are known to possess different primary structures (Hirabayashi & Perry, 1974; van Eerd & Takahashi, 1975). It is noteworthy that when the troponin C-binding site of troponin I was protected by troponin C during coupling, an affinity column containing white-skeletal-muscle troponin I was slightly more effective in removing troponin C from cardiac than from skeletal-muscle extracts. In all cases the yields of troponin C were less than quantitative values. This might be expected, as the Sepharose-bound troponin I is competing for the troponin C which is itself complexed to the troponin I present in the muscle extract.

As far as can be judged from the appearance of the CNBr digests on electrophoresis, troponin C from white and red skeletal muscle of the rabbit appear to be very similar, if not identical. Thus, unlike the case with troponin I (Syska *et al.*, 1974) and troponin T (Hitchcock, 1973; Perry & Cole, 1974), the primary structure of troponinin C apparently changes not at all or only to a limited extent with speed of contraction in skeletal muscle. The final decision as to whether the two forms of troponin C

in skeletal muscle are identical must await the results of primary-sequence studies. Rabbit cardiac troponin C is clearly different, as judged by the electrophoretic pattern of the CNBr peptides and by different behaviour in binding to troponin I–Sepharose columns.

The presence of a troponin C-like protein in smooth muscle is of particular interest, in view of the claim that the troponin complex cannot be detected in this muscle and that the Mg^{2+}-stimulated adenosine triphosphatase is solely myosin-regulated (Bremel, 1974). The troponin C-like protein that we have isolated from rabbit uterus may be similar to one of the components of 17000–18000 mol.wt. reported by Ebashi *et al.* (1975) to be present in chick gizzard muscle.

The limited studies so far carried out with the troponin C-like protein from smooth muscle indicate that this protein is very similar to its counterparts from striated muscle. That being the case, it might be expected to take part in Ca^{2+} regulation of the Mg^{2+}-stimulated adenosine triphosphatase through the actin system. An understanding of the role of troponin C in the contractile activity of smooth muscle must await further investigation of its properties.

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