The Subunits and Biological Activity of Polymorphic Forms of Tropomyosin

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1. Free thiol groups were shown to be essential for tropomyosin to effect maximum inhibition of the Ca^{2+} -stimulated ATPase (adenosine triphosphatase) of desensitized actomyosin but not for its activity in the regulatory-protein system. 2. The activity of tropomyosin on the Mg^{2+} -stimulated ATPase in the regulatory-protein system was more susceptible to enzymic digestion and thermal denaturation than its effect on the Ca^{2+} -stimulated ATPase of actomyosin. 3. Rabbit skeletal tropomyosin migrated as two distinct electrophoretic components in the presence of sodium dodecyl sulphate and urea and as four components on isoelectric focusing in urea. 4. The two main subunits present in rabbit skeletal tropomyosin, which have been named the α - and β -chains, were separated by chromatography on CM-cellulose in urea at pH 4.0. They were shown to be virtually identical in amino acid composition, except for their cysteine contents. The α_2 and β_2 forms of tropomyosin possessed all the biological activities characteristic of normal tropomyosin preparations. 5. In skeletal muscle the α and β components of tropomyosin were present in the proportion of 4:1. Somewhat lower ratios were obtained in skeletal muscle of sheep, pig and cow. 6. Tropomyosin isolated from cardiac muscle and *Pecten maximus* adductor muscle migrated as one band only. These tropomyosins possessed similar biological activities to those isolated from skeletal muscle.

The precise biological role of the myofibrillar protein tropomyosin has yet to be defined. Early suggestions that it was a precursor of myosin (Bailey, 1948) or that it might play an important role in the tonic function of striated muscle (Maréchal & Mommaerts, 1963) have not been substantiated. It is now clear, however, that tropomyosin is intimately involved in the regulation of the contraction-relaxation cycle, although much has to be learnt about the detailed mechanisms involved.

Since the discovery by Ebashi & Kodama (1966) that the troponin complex and tropomyosin were both required for the regulation of the ATPase* activity of the myofibril by changes in Ca^{2+} concentration, three effects of tropomyosin on the ATPase activity of actomyosin have been distinguished.

(1) Tropomyosin, in combination with the whole troponin complex, is generally considered to be essential for inhibition of the Mg^{2+} -stimulated ATPase of desensitized actomyosin in the absence of Ca^{2+} .

(2) Tropomyosin greatly enhances the inhibitory activity of the inhibitory protein component of the troponin complex on the Mg^{2+} -stimulated ATPase activity of desensitized actomyosin (Wilkinson *et al.*, 1972).

(3) Tropomyosin, alone inhibits the Ca^{2+} -stimulated ATPase of desensitized actomyosin (Schaub *et al.*, 1967).

The first two properties are probably closely re-

* Abbreviation: ATPase, adenosine triphosphatase.

lated and are aspects of the role of tropomyosin in the regulation of contractile activity, but the significance of the third property is not clear. As the Ca^{2+} -stimulated ATPase of myosin is not affected by tropomyosin it suggests that actin may be involved in some way in the enzymic activity of myosin when ATP is hydrolysed by actomyosin in the presence of Ca^{2+} . It also suggests that this interaction is influenced by tropomyosin. Surprisingly, however, tropomyosin alone has no effect on the Mg^{2+} -stimulated ATPase, where there is strong evidence of the interaction of actin with myosin. This effect of tropomyosin is responsible for the relatively low specific ATPase activity of myofibrils and natural actomyosin in the presence of Ca^{2+} compared with that obtained in the presence of Mg^{2+} under otherwise similar conditions.

Although the three biological properties listed above are usually associated with all preparations of tropomyosin, they can be selectively destroyed under certain conditions. Controlled proteolysis of tropomyosin greatly decreases its activity in the regulatory protein system, but does not affect its ability to inhibit the Ca^{2+} -stimulated ATPase of desensitized actomyosin (Schaub & Perry, 1969). In addition, it has been demonstrated that small amounts of a tropomyosin-like protein, which inhibits the Ca^{2+} -stimulated ATPase of desensitized actomyosin but does not restore Ca^{2+} sensitivity to the Mg^{2+} -stimulated ATPase in the presence of the troponin complex, can be obtained by SE-Sephadex chromatography of crude

troponin-complex preparations (Schaub & Perry, 1969).

Although tropomyosin has been relatively well characterized for some time, there is doubt about its precise subunit structure. The work of Woods (1966, 1967) in particular has established that the tropomyosin molecule is a dimer composed of two subunits of molecular weight about 34000. Most investigations indicate that the subunits are similar in size but little precise information has been available as to whether or not they are identical. A report by Bodwell (1967) on the separation of carboxymethylated rabbit skeletal tropomyosin into two components of different mobility and amino acid composition by preparative polyacrylamide-gel electrophoresis implied that the two subunits were not identical. Bodwell (1967) acknowledged, however, that the conditions of reduction that he used, i.e. at pH 11.5–12.0 for 12 h, may have resulted in specific cleavage of the polypeptide chain. The gross differences in mobility and composition of the fractions coupled with the known structural instability of tropomyosin at alkaline pH values (Lowey, 1965) implied that this was probably the case. More convincing evidence of heterogeneity has been obtained by electrophoresis in the presence of sodium dodecyl sulphate at pH 7.0. Weber & Osborn (1969) observed that tropomyosin migrated under these conditions on a 10% (w/v) polyacrylamide gel as a poorly resolved doublet, whereas under similar conditions but with the further addition of 5 M-urea to the gel, Sender (1971) observed two distinct bands. Further evidence of heterogeneity has come from the sequence studies of Hodges *et al.* (1972), which suggest a minimum of two and possibly four different subunit chains in tropomyosin.

The work described in the present paper was undertaken to determine whether any relationship existed between the apparent heterogeneity of tropomyosin on electrophoresis and the biological properties of different stability that are associated with tropomyosin. It is concluded that tropomyosin from skeletal muscle contains two and possibly four different subunits, which differ in cysteine content. Fractions of tropomyosin homogeneous in subunit composition possess all the biological activities normally associated with unfractionated tropomyosin. Only one type of tropomyosin could be detected in cardiac muscle. Some aspects of this work have been briefly reported (Cummins & Perry, 1972).

Methods

Preparative procedures

Unless otherwise stated, all preparations and chromatographic separations were carried out at 0–4°C.

Desensitized actomyosin. Desensitized actomyosin was prepared from natural actomyosin by the pro-

cedure described by Schaub & Perry (1971), modified as follows. After the first precipitation from the myofibrillar extract in Weber's solution (0.6 M-KCl–10 mM-Na₂CO₃–40 mM-NaHCO₃) the natural actomyosin was dissolved in 0.6 M-KCl, the pH adjusted to 7.0, and reprecipitated again by dilution with 15 vol. of water before the actomyosin was washed.

Tropomyosin. Tropomyosin was prepared from mammalian muscle by a procedure based on the method of Bailey (1948, 1951). The combined 1 M-KCl extracts from dried muscle fibre obtained as described by Bailey (1948) were acidified to pH 4.6 with 1 M-HCl and after 1 h the precipitate was separated by centrifugation. The precipitate was dispersed in 5 vol. of water, the pH readjusted to 7 with 1 M-NaOH and the solution was stirred slowly for 1 h, after which time any material that remained insoluble was discarded. The solution was then salted out between 40% and 70%-satd. (NH₄)₂SO₄ by using satd. (NH₄)₂SO₄ solution which had been adjusted to pH 7 with conc. NH₃ (sp.gr. 0.880). After centrifugation, the protein precipitate was dialysed exhaustively against 10 mM-Tris-HCl buffer, pH 7.6. Isoelectric precipitation at pH 4.6 in the presence of 1 M-KCl followed by (NH₄)₂SO₄ fractionation at pH 7.0 between 53% and 60% saturation was then carried out three times. Throughout these purification cycles, the protein solutions were adjusted to approx. 1 mg/ml by using an $E_{280}^{1\%}$ value for tropomyosin of 3.3 (Woods, 1969) to minimize co-precipitation of the troponin complex (Hartshorne & Mueller, 1969).

Occasionally, tropomyosin was also prepared by the method of Hartshorne & Mueller (1969) or by recovery from the isoelectric precipitate obtained at pH 4.6 during the preparation of troponin complex (Schaub & Perry, 1969). In all cases it was subjected to repeated cycles of isoelectric precipitation and (NH₄)₂SO₄ fractionation as described above. In some cases tropomyosin was also prepared with the addition of 15 mM-2-mercaptoethanol or 0.5 mM-dithiothreitol to all solutions. Finally tropomyosin preparations were exhaustively dialysed against water, freeze-dried and stored at –20°C.

All tropomyosin preparations were examined by polyacrylamide-gel electrophoresis and for their effect on the Mg²⁺-stimulated ATPase activity of desensitized actomyosin in the presence and absence of EGTA [ethanedioxybis(ethylamine)tetra-acetate] to ensure that they were free of contamination by components of the troponin complex.

Tropomyosin from the whole adductor muscle of the mollusc *Pecten maximus* was prepared by the method of Bailey (1956, 1957) and Bailey & Ruegg (1960).

Tropomyosin-like fraction present in crude troponin complex. This protein fraction was prepared by SE-Sephadex C-50 chromatography of crude troponin complex as described by Schaub & Perry (1969).

Troponin complex and components. Troponin complex was prepared from the low-ionic-strength extract of myofibrils possessing regulatory-protein-system activity, by the method of Schaub & Perry (1969) and also occasionally by the method of Ebashi *et al.* (1971). After exhaustive dialysis against 10mM-Tris-HCl buffer (pH 7.6)–0.5mM-dithiothreitol, preparations were freeze-dried and stored at -20°C .

The inhibitory protein and 37000-molecular-weight components were prepared from inhibitory-factor preparations (Schaub & Perry, 1971) either by the method of Wilkinson *et al.* (1972) or by a method based on the procedure of Greaser & Gergely (1971). In the latter case inhibitory-factor preparations were applied to a column of DEAE-Sephadex ($30\text{cm} \times 2\text{cm}$) equilibrated against 50mM-Tris-HCl buffer (pH 8.0)–6mM-urea–1mM-dithiothreitol. On application of a linear gradient of 0–0.6M-KCl in 600ml of buffer, two peaks corresponding to the inhibitory protein and 37000-molecular-weight component respectively were obtained. The fractions represented by these peaks were rechromatographed until homogeneous as judged by polyacrylamide-gel electrophoresis in sodium dodecyl sulphate (Weber & Osborn, 1969).

Assay of ATPase activity

ATPase activity was assayed as described by Schaub & Perry (1969). The specific activities at 25°C of the desensitized actomyosin preparations, expressed as μg of $\text{P}_i/5\text{min}$ per mg of protein, varied between 70 and 90 in the presence of MgCl_2 and 80 and 100 in the presence of CaCl_2 .

Papain digestion

Insoluble papain prepared by the method of Cebra *et al.* (1961) and dissolved in 5mM-cysteine-HCl–2mM-EDTA, pH 6.0, was used at tropomyosin/papain ratios of 40:1–60:1 (w/w). Digestion was carried out in 20mM-Tris-HCl buffer (pH 7.6)–15mM-2-mercaptoethanol at 25°C and terminated by removing the insoluble papain by centrifugation for 5min.

Chromatography of tropomyosin

Whatman CM-cellulose (CM-52; microgranular; pre-swollen form) was equilibrated with 50mM-sodium formate buffer (pH 4.0)–8mM-urea–15mM-2-mercaptoethanol and packed into a glass column ($12\text{cm} \times 1.5\text{cm}$). Tropomyosin (5–10mg) in 2ml of column buffer was applied to the column and eluted by a linear gradient of 0–0.2M-NaCl in 200ml of buffer. Elution was effected with a 10–20cm head of buffer at a flow rate of 5ml/h and 1ml fractions were collected. The fractions were dialysed against

10mM-Tris-HCl buffer (pH 7.6)–0.5mM-dithiothreitol and freeze-dried.

Electrophoresis

Polyacrylamide-gel electrophoresis was performed in glass tubes ($6\text{mm} \times 80\text{mm}$) in an apparatus built as described by Davies (1964) and also in vertical ($6\text{cm} \times 20\text{cm} \times 0.2\text{cm}$) slab assemblies as described by Perrie *et al.* (1973).

Urea system. This was carried out at pH 8.5 as described by Schaub & Perry (1969) with 6M-urea–25mM-Tris–80mM-glycine buffer (pH 8.5)–15mM-2-mercaptoethanol in the gels.

Sodium dodecyl sulphate system. This was carried out in general as described by Weber & Osborn (1969): 10% (w/v) polyacrylamide gels were used throughout. Occasionally sodium phosphate buffer, pH 7.0, was replaced by 82.5mM-Tris–400mM-boric acid buffer, pH 7.0. Urea (5M) was included in the gels as indicated in the text.

Densitometric scanning of the gels was carried out with a Gilford model 2400 recording spectrophotometer fitted with a Gilford model 2410 gel-scanning attachment. The areas beneath the densitometrically traced peaks were calculated by counting the number of squares after superimposition on graph paper, or by multiplying the height of the peaks by the width at the half height.

Isoelectric focusing

Polyacrylamide gel. Isoelectric focusing in polyacrylamide gels was carried out at 4°C in general by the methods of Righetti & Drysdale (1971) and Vesterberg (1972) by using the slab assembly as described above. The final concentrations of reagents in the gels were 4% acrylamide, 0.16% *NN'*-methylenebisacrylamide, 0.02% *NNN'*-tetramethylethylenediamine, 0.02% ammonium persulphate, 6M-urea and 2.0% (w/v) ampholyte buffer. The gel solutions were de-aerated and left for 30–60min to set.

The electrode vessels were filled with solutions of 0.01M- H_3PO_4 and 0.02M-NaOH as anolyte and catholyte respectively, the latter being uppermost. After electrophoresis at 10mA/slab for 10min to remove excess of ammonium persulphate, samples were applied to the top of the gels. A sample (10–20 μl) containing 10–20 μg of protein in 2% ampholytes and 6M-urea titrated to pH 9.0 with 1M-NaOH was normally applied. Electrophoresis was performed at a constant current (10mA) until the voltage had risen to 400V. The voltage was then maintained at this value for 6h. Gels were stained with Coomassie Brilliant Blue R (George T. Gurr, Searle Scientific Services, High Wycombe, Bucks.,

U.K.) in a solution containing methanol, sulphosalicylic acid and trichloroacetic acid at 60°C for 15 min as described by Vesterberg (1972). The destaining solution consisted of acetic acid-ethanol-water (2:5:13, by vol.) and was replaced every 20 min until destaining was complete.

Sucrose density gradient. Isoelectric focusing in sucrose density gradients was carried out by using the procedure of Haglund (1967) in a 100 ml electrofocusing column (LKB Producter, Bromma 1, Sweden). Sulphuric acid (1.6M) and ethylenediamine 2.0% (v/v) were used in the anode and cathode electrode solutions respectively and 7M-urea and 4.2% polyoxyethylene dodecyl ether (BRIJ 35, a non-ionic detergent) were incorporated into a 9.4–43.5% sucrose density gradient. For the first electrofocusing run, the ampholyte buffer concentration was 4.0%. For the second run in which the focused protein fraction was pooled (approx. 24 ml), the ampholyte concentration was approx. 1%, and the urea, sucrose and BRIJ 35 concentrations were identical with those used in the preliminary run.

Determination of protein concentration

Protein was determined as described by Schaub & Perry (1971) assuming an N content of 16%.

Reduction and carboxymethylation of proteins

Proteins were reduced and *S*-carboxymethylated with iodoacetic acid at pH 8.6 by the procedure of Crestfield *et al.* (1963), except that urea was replaced by specially purified 6M-guanidine hydrochloride (British Drug Houses Ltd., Poole, Dorset, U.K.).

Radioactivity counting

Either 0–50 μ l or exactly 1 ml of aqueous solution containing iodo[14 C]acetate-labelled protein in 20 ml glass scintillation vials was added to 9.0 ml of toluene-Triton X-100 (2:1, v/v). The toluene contained 4 g of 2,5-diphenyloxazole and 50 mg of 1,4-bis-(5-phenyloxazol-2-yl)benzene per litre. Radioactive counting was carried out in a Philips Model A liquid-scintillation counter with automatic subtraction of background radioactivity.

Amino acid analysis

Amino acid analysis was in general performed with the Beckman 120-B automatic amino acid analyser as described by Wilkinson *et al.* (1972). Cysteine was determined as *S*-carboxymethylcysteine on the same chromatographic column. Tryptophan was determined by reaction with *p*-dimethylaminobenzaldehyde and oxidation with NaNO₂ as described by Spies & Chambers (1948, 1949).

Reagents

Sodium dodecyl sulphate was obtained from Sigma (London) Chemical Co. Ltd., London S.W.6, U.K. Papain (twice recrystallized) was supplied by Worthington Biochemical Corp., Freehold, N.J., U.S.A., iodo[2- 14 C]acetate was from the Radiochemical Centre, Amersham, Bucks., U.K., and polyoxyethylene dodecyl ether (BRIJ 35) was from Honeywell and Stein Ltd., London W.1, U.K.

Results

Biological activity of tropomyosin and tropomyosin-like preparations

Blocking of thiol groups. Both untreated and carboxymethylated tropomyosin were able to inhibit the Mg²⁺-stimulated ATPase of desensitized actomyosin by up to 80% when tested in the presence of 1 mM-EGTA and optimum amounts of troponin complex (Fig. 1). Usually no significant loss of activity in this system could be detected after carboxymethylation of tropomyosin. Occasionally, however, carboxymethylation caused some enhancement of inhibitory activity, but this never exceeded about 10%. Carboxymethylation also had no effect on the property of enhancing the inhibitory activity of the inhibitory protein on the Mg²⁺-stimulated ATPase of desensitized actomyosin (Wilkinson *et al.*, 1972; Perry *et al.*, 1972). When tested alone, between 30 and 50 μ g of the inhibitory protein produced up to 20% inhibition of the Mg²⁺-stimulated ATPase of 1 mg of desensitized actomyosin. Addition of carboxymethylated or untreated tropomyosin to the system increased the inhibition up to a similar value in each case, i.e. up to 80%. This inhibition was insensitive to Ca²⁺.

In contrast, treatment with thiol reagents selectively decreased the inhibitory action of tropomyosin on the Ca²⁺-stimulated ATPase of desensitized actomyosin. For example, concentrations of tropomyosin that inhibited the Ca²⁺-stimulated ATPase by about 60% produced only 20% inhibition after carboxymethylation (Fig. 1). The decrease in the effectiveness of tropomyosin in inhibiting the Ca²⁺-stimulated ATPase was also obtained after blocking the thiol groups with phenylmercuric acetate. This reagent had no effect on the biological activity of tropomyosin in the troponin system.

The loss of inhibition of the Ca²⁺-stimulated ATPase by tropomyosin that had been treated with thiol reagents was reversible. After reaction with a 200-fold molar excess of bis-2-carboxyethyl disulphide (Stracher, 1964) at pH 9.3 for 90–100 h, tropomyosin was only 20% as effective as the untreated control in inhibiting the Ca²⁺-stimulated ATPase (Fig. 2). Its ability to inhibit the Mg²⁺-stimulated ATPase in the presence of troponin complex or inhibitory protein remained unaffected. After 144 h of treatment with

Fig. 1. Effect of carboxymethylation on the biological activity of tropomyosin

Tropomyosin was carboxymethylated as described in the Methods section and enzymic assays were carried out under standard assay conditions in 25 mM-Tris-HCl buffer, pH 7.6, with 2.5 mM-ATP and 0.7 mg of desensitized actomyosin and the additions indicated. ATPase activity is expressed as a percentage of that in the presence of all additions, except tropomyosin in each system. Δ , $\circ$, Untreated tropomyosin; $\blacktriangle$, $\bullet$, carboxymethylated tropomyosin. (a) Mg²⁺-stimulated ATPase activity: 86 μ g of troponin complex, 2.5 mM-MgCl₂ and 1 mM-EGTA; (b) Ca²⁺-stimulated ATPase activity; 2.5 mM-CaCl₂.

bis-2-carboxyethyl disulphide the tropomyosin was dialysed against 15 mM-2-mercaptoethanol-20 mM-Tris-HCl buffer, pH 9.3. The recovery in the ability of the disulphide-exchanged tropomyosin to inhibit the Ca²⁺-stimulated ATPase was essentially complete after 10 h of dialysis under these conditions (Fig. 3).

Heat denaturation. The ability of tropomyosin to inhibit the Ca²⁺-stimulated ATPase of desensitized actomyosin was decreased by 20% after heating at 60°C for 70 min at concentrations of 0.3–0.5 mg/ml in 20 mM-Tris-HCl buffer (pH 7.6)–15 mM-2-mercaptoethanol. In contrast, its ability to inhibit the Mg²⁺-stimulated ATPase in the presence of troponin complex and EGTA was decreased by over 80% after the same treatment (Fig. 4). Similar results were obtained when tropomyosin was heated under the same conditions for 10 min at 100°C. This selective loss in the biological activity of tropomyosin in the presence of the troponin complex was observed when tropomyosin preparations were heated in the pH range 2–9 and at ionic strengths ranging from 0.02 to 1.1.

Enzymic digestion. Earlier studies (Schaub & Perry, 1969) have shown that the ability of tropomyosin to restore regulatory-protein-system activity to the troponin complex can be selectively destroyed by mild tryptic digestion, leaving its inhibitory effect on the Ca²⁺-stimulated ATPase virtually unchanged. Selective destruction of the biological activity of tropo-

myosin was also obtained by digestion with papain. After 30 min incubation with insoluble papain, tropomyosin preparations almost completely lost their ability to inhibit the Mg²⁺-stimulated ATPase in the presence of troponin complex and EGTA (Fig. 5), but were still 50% as active as the undigested controls in inhibiting the Ca²⁺-stimulated ATPase of actomyosin.

Tropomyosin-like fraction obtained by SE-Sephadex chromatography. The material present in crude troponin complex that was not retained by chromatography on SE-Sephadex in 33 mM-sodium citrate buffer (pH 6.0)–1 mM-dithiothreitol migrated at pH 8.5 in the presence of urea with a mobility identical with that of tropomyosin. It also inhibited the Ca²⁺-stimulated ATPase of desensitized actomyosin but did not, however, confer regulatory-protein-system activity on the troponin complex (Schaub & Perry, 1969).

The E_{278}/E_{260} ratio of the first peak eluted from SE-Sephadex by 33 mM-sodium citrate buffer (pH 6.0)–1 mM-dithiothreitol was 1.12 ± 0.1 (cf. Schaub & Perry, 1969), compared with a value of 2.09 for the standard tropomyosin preparations. This latter value was unchanged after SE-Sephadex chromatography (see the Methods section). When the material present in the first peak was subjected to the isoelectric-precipitation and (NH₄)₂SO₄-fractionation-purification cycles outlined in the Methods section, the

Fig. 2. Effect of disulphide-exchanged tropomyosin on the Mg^{2+} - and Ca^{2+} -stimulated ATPase activities of desensitized actomyosin

Tropomyosin (2.3 mg/ml) was treated with a 200-fold molar excess of bis-2-carboxyethyl disulphide in 20 mM-Tris-HCl buffer, pH 9.3, at 4°C. Samples were taken at the times indicated and enzymic assays carried out in 25 mM-Tris-HCl buffer, pH 7.6, and 2.5 mM-ATP, on 0.7 mg of desensitized actomyosin, with the additions indicated. Relative activity is expressed as a percentage of the inhibition of the ATPase obtained with untreated tropomyosin in each system. ▲, Mg^{2+} -stimulated ATPase activity: 29 μ g of inhibitory protein, 2.5 mM- $MgCl_2$, 1 mM-EGTA and 87.5 μ g of treated tropomyosin. ●, Ca^{2+} -stimulated ATPase activity: 2.5 mM- $CaCl_2$ and 87.5 μ g of treated tropomyosin.

$E_{278}^{1\%}$ value rose to 3.08 ± 0.1 and the E_{278}/E_{260} ratio to 1.98 ± 0.2 . These values are in close agreement with those of normal tropomyosin (Woods, 1969).

The presence in the first fraction eluted from SE-Sephadex of lower-molecular-weight material in addition to that migrating with a similar mobility to tropomyosin was demonstrated by polyacrylamide-gel electrophoresis in sodium dodecyl sulphate. After three cycles of isoelectric precipitation and $(NH_4)_2SO_4$ fractionation as used in the preparation of tropomyosin these components were removed and the electrophoretic pattern corresponded to that of tropomyosin. At low protein loadings (5–10 μ g), the two closely spaced bands that were a feature of normal tropomyosin preparations could also be observed (see below).

The amino acid composition of the purified first-

Fig. 3. Effect of 2-mercaptoethanol treatment of disulphide-exchanged tropomyosin on the Ca^{2+} -stimulated ATPase of desensitized actomyosin

Tropomyosin (2.1 mg/ml) that had been treated with bis-2-carboxyethyl disulphide as described in Fig. 2 was dialysed against 15 mM-2-mercaptoethanol–20 mM-Tris-HCl buffer, pH 9.3 at 4°C. Samples were taken at the times indicated and enzymic assays carried out in 25 mM-Tris-HCl buffer (pH 7.6)–2.5 mM- $CaCl_2$ –2.5 mM-ATP with 0.70 mg of desensitized actomyosin and 87.5 μ g of tropomyosin. Relative ATPase activity is expressed as in Fig. 2.

peak fraction is given in Table 1. The lack of tryptophan, the very low proline content and the very similar overall composition indicates that this fraction is closely related to, if not identical with, tropomyosin.

All the first-peak fractions used in this study were tested for inhibition of the ATPase activity of desensitized actomyosin. Between 20 and 40 μ g of the purified fraction were required to obtain 50% of the maximum inhibition of the Ca^{2+} -stimulated ATPase of 1 mg of desensitized actomyosin compared with about 20 μ g for normal tropomyosin preparations. Moreover, after carboxymethylation, the ability of the first-peak fractions to inhibit the Ca^{2+} -stimulated ATPase was considerably decreased. Neither the crude nor the purified first-peak fractions were able to inhibit the Mg^{2+} -stimulated ATPase in the presence of optimum amounts of troponin complex and EGTA.

Isolation and properties of α and β tropomyosins

Under the standard conditions of electrophoresis on 8.0% (w/v) polyacrylamide in 25 mM-Tris–80 mM-

Fig. 4. Effect of heating tropomyosin at 60°C on the ATPase activity of desensitized actomyosin

Tropomyosin (4.44 mg/ml) was heated at 60°C for the times indicated in 20 mM-Tris-HCl buffer (pH 7.6)–15 mM-2-mercaptoethanol. Enzymic assays were carried out under standard conditions on 0.65 mg of desensitized actomyosin. Relative activity with heated tropomyosin is expressed as in Fig. 2. ●, ATPase activity in the presence of 89 μ g of tropomyosin, 70 μ g of troponin complex, 2.5 mM-MgCl₂ and 1 mM-EGTA; ▲, ATPase activity in the presence of 89 μ g of tropomyosin and 2.5 mM-CaCl₂.

glycine buffer, pH 8.5, tropomyosin did not migrate into the gel unless a dissociating agent was present. On the addition of 6–8 M urea to the gel all tropomyosin preparations obtained from rabbit skeletal muscle migrated as two closely spaced bands (Plate 1a), which were quite distinct when 5–10 μ g was loaded on the gel but were not resolved if 30–40 μ g of tropomyosin was applied. The faster of the two bands observed on electrophoresis at this pH value was the major component. The relative amounts of the two bands, which were in the ratio of about 4:1, did not change significantly after repeated attempts to further purify tropomyosin preparations by precipitation with (NH₄)₂SO₄ over narrow ranges of concentration (53–58%, 54–59%, and 55–60% saturation) and at known protein concentrations (0.25–2.0 mg/ml). Likewise the ratios of the amounts of protein in the two bands were similar to those present in tropomyosin recovered from the isoelectric precipitate

Fig. 5. Effect of digestion with papain on the biological properties of tropomyosin

Tropomyosin (3.2 mg/ml) was digested with insoluble papain at tropomyosin/papain ratios of 40:1–60:1 (w/w) in 20 mM-Tris-HCl buffer (pH 7.0)–15 mM-2-mercaptoethanol for the times indicated. Digestion was stopped by sedimentation of the insoluble papain. Enzymic assays were carried out under standard conditions with 2.5 mM-ATP on 0.63 mg of desensitized actomyosin. The effects of papain treatment are expressed as the percentage of inhibition of the ATPase in each system obtained with the digested tropomyosin compared with the untreated control. ▲, ●, ■, Mg²⁺-stimulated ATPase activity with three different tropomyosin preparations: 70–90 μ g of tropomyosin, 100–120 μ g of troponin complex, 2.5 mM-MgCl₂ and 1 mM-EGTA; △, ○, □, Ca²⁺-stimulated ATPase activity of the same preparations: 90–110 μ g of tropomyosin, 2.5 mM-CaCl₂.

obtained at pH 4.6, during the preparation of troponin complex. This latter procedure did not involve the use of organic solvents, in contrast with the standard methods for tropomyosin preparation.

The double-banded appearance was not due to partial aggregation resulting from oxidation of thiol groups, for it was also obtained when electrophoresis was carried out with fully reduced tropomyosin in the presence of 15 mM-2-mercaptoethanol or 1 mM-dithiothreitol. After carboxymethylation of tropomyosin by treatment with iodoacetate a similar two-band electrophoretic pattern was observed.

Table 1. *Amino acid composition of purified first-peak fractions eluted from SE-Sephadex on chromatography of crude troponin complex*

For experimental details see the text. The values given are the means of four preparations expressed as amino acid residues/68 000 g of protein.

Amino acid	Amino acid residues/68 000 g of protein	
	First-peak fraction	Tropomyosin from rabbit skeletal muscle
Lys	79.0	78.5
His	4.7	4.4
Arg	28.4	30.7
Asx	63.0	63.8
Thr	14.3	16.7
Ser	27.8	28.0
Glx	148.0	149.5
Pro	0.8	0.5
Gly	9.3	8.9
Ala	77.1	76.4
Val	21.0	20.2
Met	11.8	11.7
Ile	27.3	23.9
Leu	72.3	68.0
Tyr	11.4	10.7
Phe	2.0	2.9
Cys	3.0	2.7
Tyr	0	0

In addition to the possible differences in charge suggested by the electrophoretic pattern obtained in urea the two components appeared to have different molecular weights. A band pattern similar to that seen in the presence of urea was observed both with native and carboxymethylated tropomyosin on electrophoresis in 0.1% sodium dodecyl sulphate in 82.5 mM-Tris-400 mM-boric acid buffer, pH 7.0 (Plate 1a). Two bands in relative amounts corresponding to those found for isolated tropomyosin were also observed when urea extracts of whole myofibrils were subjected to electrophoresis in the presence of sodium dodecyl sulphate. The separation on sodium dodecyl sulphate electrophoresis in the presence of 0.1 M-sodium phosphate buffer, pH 7.0 (Weber & Osborn, 1969) was much less satisfactory than that obtained in the Tris-borate buffer. In the latter system values of 32 000 and 36 000 were obtained for the apparent molecular weights of the major and minor components respectively.

It has been reported that the troponin-T component of the troponin complex, which corresponds to the 37 000 molecular-weight component obtained by Wilkinson *et al.* (1972) in this laboratory, is often associated with tropomyosin preparations (Greaser

& Gergely, 1971). Samples of the 37 000-molecular-weight component were therefore added to the tropomyosin and sodium dodecyl sulphate gel electrophoresis was carried out on the mixture. In Tris-borate buffer systems the 37 000-molecular-weight component migrated more slowly than either of the two components present in tropomyosin, although it was not well resolved from the tropomyosin in this system. Non-identity of the 37 000-molecular-weight component with either of the tropomyosin components was confirmed by sodium dodecyl sulphate electrophoresis in the presence of urea (Sender, 1971). Under these conditions the electrophoretic mobility of tropomyosin was greatly decreased whereas the mobilities of the other recognized myofibrillar components were unaffected. In 82.5 mM-Tris-400 mM-boric acid buffer, pH 7.0, containing 0.1% sodium dodecyl sulphate and 5 M-urea, the tropomyosin migrated as two bands, both slower than that corresponding to the 37 000-molecular-weight component, with apparent molecular weights of 51 000 and 53 000 for the major and minor components respectively (Plate 1b).

Isoelectric focusing of tropomyosin preparations

When isoelectric focusing was carried out in a sucrose gradient in 1% (w/v) Ampholine buffers in the pH range 3-6 (Haglund, 1967) a band of turbidity was observed that could not be resolved into fractions on elution. To prevent precipitation of the tropomyosin at its isoelectric point, focusing was carried out in the presence of 7 M-urea (Bloemendal & Schoenmakers, 1968; Björk, 1968) and BRIJ-35 (polyoxyethylene dodecyl ether) (Godson, 1970; Frieson *et al.*, 1971) a non-ionic detergent. Normal and [^{14}C]carboxymethylated tropomyosin were focused in 4% ampholytes in the pH range 4-6, and the protein was eluted and refocused, thereby producing a pH gradient from 5.0 to 5.5. In this system normal tropomyosin was eluted at pH 5.1 and [^{14}C]carboxymethylated tropomyosin at pH 5.3. Although there was some evidence of heterogeneity in the elution profile, the components could not be separated in amounts adequate for analysis owing to the poor resolution of the peaks on elution.

Satisfactory stabilization of the bands was obtained by isoelectric focusing in 4% (w/v) polyacrylamide gels (Righetti & Drysdale, 1971; Vesterberg, 1972) in 2% Ampholine buffers. Under these conditions, two main bands with relative intensities similar to those observed in urea and sodium dodecyl sulphate electrophoresis were observed (Plate 2a). In addition, two very much fainter fine satellite bands immediately behind the main components were consistently observed when the range of Ampholine buffers was restricted to pH 4-6.

EXPLANATION OF PLATE 1(a)

Polyacrylamide-gel electrophoresis of rabbit skeletal-muscle tropomyosin

(A) and (B) show 8% (w/v) gel with 6M-urea, pH 8.5 buffer (see the Methods section); (C) and (D) show 10% (w/v) gel with 0.1% sodium dodecyl sulphate and 82.5 mM-Tris–400 mM-boric acid buffer, pH 7.0. (A) 5 μ g of tropomyosin; (B) 5 μ g of carboxymethylated tropomyosin; (C) 30 μ g of tropomyosin; (D) 30 μ g of carboxymethylated tropomyosin.

EXPLANATION OF PLATE 1(b)

Polyacrylamide-gel electrophoresis of tropomyosins and the 37000-molecular-weight component

Fully reduced and carboxymethylated tropomyosins and the 37000-molecular-weight component of the troponin complex were subjected to electrophoresis under standard conditions in Tris–borate buffer, pH 7.0, in the presence of 0.1% sodium dodecyl sulphate and 5M-urea. (A) 15 μ g of rabbit skeletal-muscle tropomyosin; (B) 10 μ g of 37000-molecular-weight component; (C) 15 μ g of rabbit skeletal-muscle tropomyosin + 10 μ g of 37000-molecular-weight component; (D) 15 μ g of rabbit cardiac-muscle tropomyosin; (E) 15 μ g of *Pecten maximus* adductor tropomyosin.

EXPLANATION OF PLATE 2(a)

Isoelectric focusing of rabbit skeletal-muscle tropomyosin

Samples (10 μ g) of fully reduced and carboxymethylated tropomyosin and the α and β fractions obtained by chromatography as described in Fig. 6 were electrofocused in 4% (w/v) polyacrylamide gel in 2% (w/v) Ampholine buffers in the pH range 4–6 containing 6 M-urea. (A) Carboxymethylated tropomyosin; (B) β fraction of carboxymethylated tropomyosin; (C) α fraction of carboxymethylated tropomyosin.

EXPLANATION OF PLATE 2(b)

Polyacrylamide-gel electrophoresis of tropomyosins isolated from different species and muscle types

All samples of tropomyosin were fully reduced and carboxymethylated. Electrophoresis was carried out in the presence of 0.1% sodium dodecyl sulphate, in Tris–borate buffer, pH 7.0 (see the Methods section) with 20 μ g samples of: (A) rabbit skeletal tropomyosin; (B) rabbit cardiac tropomyosin; (C) *Pecten maximus* adductor tropomyosin; (D) sheep skeletal tropomyosin; (E) cow skeletal tropomyosin; (F) pig skeletal tropomyosin.

Fig. 6. Chromatography of ^{14}C -labelled rabbit skeletal-muscle tropomyosin on CM-cellulose

A sample (6 mg) of fully reduced and ^{14}C carboxymethylated rabbit skeletal-muscle tropomyosin was applied to a column (20 cm $\times$ 1.5 cm) of CM-cellulose equilibrated against 50 mM-sodium formate buffer (pH 4.0)–8 M-urea. A linear gradient of NaCl from 0–0.2 M was applied after 20.0 ml had been eluted. Fractions (1 ml) were collected, from which 50 μl samples were used for measurement of radioactivity. $\bullet$, E_{280} ; $\circ$, radioactivity (d.p.m.); Δ , NaCl concentration.

Separation of tropomyosin components

A number of procedures in addition to isoelectric focusing, including gel filtration on Sephadex G-200 in the presence and absence of urea, guanidine hydrochloride and sodium dodecyl sulphate, and chromatography on DEAE-cellulose and Amberlite CG-50, were used in an attempt to separate the two components of rabbit skeletal-muscle tropomyosin. None of these proved to be satisfactory. The method ultimately adopted was based on an unpublished procedure developed in this department by Dr. J. M. Wilkinson for the purification of the inhibitory protein and the 37000-molecular-weight component of the troponin complex (below and Fig. 6). When a mixture of these two proteins that also contained tropomyosin was chromatographed on CM-cellulose at pH 4.0, tropomyosin was eluted before both the inhibitory protein and the 37000-molecular-weight component, and it was noted on subsequent electrophoretic examination that some fractionation of the two main components of tropomyosin was obtained during its elution.

When fully reduced tropomyosin was applied to a column of CM-cellulose equilibrated with 50 mM-sodium formate buffer (pH 4.0)–8 M-urea–15 mM-2-mercaptoethanol the tropomyosin was retained on the column. It was subsequently eluted in two peaks when a gradient of 0–0.2 M-NaCl was applied (Fig. 6). The first peak corresponded to the minor component of slower mobility observed on electrophoresis in urea and sodium dodecyl sulphate and the second and larger peak to the major electrophoretic component. Each peak was shown by isoelectric focusing on polyacrylamide gel to contain the minor or satellite component of slightly lower mobility than each of

the two main bands seen on isoelectric focusing of the whole complex (Plate 2a).

As the major components present in both peaks possessed the properties of tropomyosin (see below) they were considered to represent different forms of this protein and were named α and β fractions in descending order of mobility on electrophoresis in urea at pH 8.5. Thus the main electrophoretic component of the first peak eluted from CM-cellulose and which corresponded to the slower of the two major bands seen on isoelectric focusing in polyacrylamide gels (Plate 2a) was the β^1 component. Likewise the major component present in the second peak and which represented the major component of tropomyosin from skeletal muscle was the α^1 component (see the Discussion section for further comments on nomenclature).

Amino acid analysis

The proteins present in the α and β fractions obtained by chromatography of reduced or carboxymethylated tropomyosin on CM-cellulose were very similar in amino acid composition (Table 2). Except for the cysteine content, which was approximately twice as great in the β as in the α fraction of tropomyosin, the values for all the residues were sufficiently close to fall within the range of experimental error expected between samples of identical amino acid composition.

Cysteine content

The amino acid analyses of the separated α and β forms of carboxymethylated tropomyosin suggested that these two components contained one and two

Table 2. *Amino acid composition of tropomyosins*

Analyses are expressed as residues/68 000 g of tropomyosin. Values are means of the number of preparations indicated.

Amino acid	Amino acid residues/68 000 g of tropomyosin							
	Rabbit skeletal			Rabbit cardiac	<i>Pecten maximus</i> adductor	Sheep skeletal	Pig skeletal	Cow skeletal
	Unfractionated	α -component	β -component					
Lys	78.5	78.1	78.6	81.0	60.8	75.7	83.7	78.7
His	4.4	4.2	4.2	4.3	0	5.3	5.1	5.1
Arg	30.7	30.9	31.0	30.3	33.9	31.8	30.5	32.1
Asx	63.8	63.0	64.1	62.9	83.8	62.1	61.4	57.6
Thr	16.7	16.2	16.7	16.2	39.2	17.8	16.9	16.1
Ser	28.0	28.4	28.0	26.5	24.0	25.5	25.9	23.9
Glx	149.5	148.9	148.7	150.0	146.9	146.1	143.8	147.7
Pro	0.5	0.3	0.3	1.1	0.3	2.9	2.5	2.0
Gly	8.9	9.9	9.1	9.7	10.2	13.4	12.4	12.5
Ala	76.4	75.8	76.4	76.7	70.1	71.4	71.1	72.0
Val	20.2	20.2	21.0	21.8	26.7	24.1	22.8	22.0
Met	11.7	10.9	11.9	13.0	8.3	15.6	15.2	16.5
Ile	23.9	23.6	23.6	23.7	17.0	23.0	22.7	20.8
Leu	68.0	68.0	68.0	65.5	64.7	61.1	62.8	65.4
Phe	2.9	2.9	2.8	3.3	7.9	6.1	5.6	4.4
Cys	2.7	1.7	3.8	2.1	1.8	2.9	2.6	3.0
Tyr	10.7	10.3	10.7	11.7	7.4	10.4	13.0	11.1
No. of preparations ...	8	2	2	3	2	2	2	2

residues of cysteine/34 000 g of tropomyosin respectively. A similar ratio of cysteine content between the two fractions was indicated by determination of the specific radioactivity of the two major components separated by polyacrylamide-gel electrophoresis of tropomyosin carboxymethylated with iodo[2- 14 C]-acetate. The bands obtained on electrophoresis in the presence of 0.1% sodium dodecyl sulphate in 82.5 M-Tris-400 mM-boric acid buffer, pH 7.0, were cut out and the relative amounts of protein and radioactivity determined. Whereas the amount of protein present in the β fraction was one-quarter of that in the α fraction its radioactivity was about one-half (Table 3). This implied that the specific radioactivity of the α component was approximately half that of the β component.

When [14 C]carboxymethylated tropomyosin was fractionated on CM-cellulose (Fig. 6) the ratio of specific radioactivity of the β fraction to that of the α fraction was 2.09:1.

Biological activity

Two preparations of highly purified tropomyosin that had been fully reduced under standard conditions at pH 8.6, and which possessed all three biological

activities were fractionated into the α and β components as illustrated in Fig. 6.

The separated components were each able to inhibit the Mg^{2+} -stimulated ATPase activity of desensitized actomyosin in the presence of troponin complex and EGTA by up to 60% compared with a maximum inhibition of 70–80% obtained with the unfractionated tropomyosin (Fig. 7). Both components were equally effective on a weight basis although 20–30% more protein was required to effect a half-maximal inhibition than with unfractionated tropomyosin. When the α and β components were assayed together in a ratio of 4:1 (w/w) they were only marginally more effective than when tested individually. Essentially similar results were obtained when the α and β components were tested for their ability to enhance the activity of the inhibitory protein on the Mg^{2+} -stimulated ATPase of desensitized actomyosin.

When tested both separately and in combination on the Ca^{2+} -stimulated ATPase activity of desensitized actomyosin, the α and β components were equally active in effecting a 35–45% inhibition of the enzymic activity (Fig. 7). The components were almost as effective as the unfractionated tropomyosin, only slightly greater amounts being required to effect a half-maximum inhibition.

Table 3. Ratios of amounts of protein and carboxymethylcysteine present in the α and β fractions of skeletal-muscle tropomyosins

Tropomyosin carboxymethylated with iodo[2- 14 C]acetate was subjected to electrophoresis under standard conditions on polyacrylamide gel in the presence of sodium dodecyl sulphate and Tris-borate buffer, pH 7.0. Protein ratios were determined by densitometric gel scanning assuming that both components bound equivalent amounts of dye. Numbers of determinations are given in parentheses.

Muscle type	Ratio of β/α		
	Protein	Radioactivity (d.p.m.)	Specific radioactivity
Rabbit (4)	1:3.83	1:1.85	2.06:1
Sheep (2)	1:1.31	1:0.66	1.98:1
Cow (2)	1:1.57	1:0.79	1.98:1
Pig (2)	1:1.78	1:0.92	1.93:1

Fig. 7. Effect of the α and β components of tropomyosin on the ATPase activity of desensitized actomyosin

The α and β components obtained as indicated in the text were tested under standard conditions in the presence of 2.5 mM-ATP on 0.73 mg of desensitized actomyosin with the additions indicated. Relative ATPase activity is expressed as for Fig. 1. (a) Mg^{2+} -stimulated ATPase activity; 91 μ g of troponin complex, 1 mM-EGTA and 2.5 mM- $MgCl_2$; (b) Ca^{2+} -stimulated ATPase activity; 2.5 mM- $CaCl_2$. ●, α component; ○, β component; ▲, mixture of α and β components (4:1, w/w); △, unfractionated tropomyosin.

Tropomyosins isolated from different species and muscle types

The two-band appearance of rabbit skeletal tropomyosin on electrophoresis in sodium dodecyl sulphate was a consistent feature of preparations from skeletal muscle, for it was also observed on electrophoresis of tropomyosin preparations isolated from skeletal muscles of the sheep, pig and cow (Plate 2b). Tropomyosin from skeletal muscle of the rabbit made dystrophic by deprivation of vitamin E (see, Lobley

et al., 1971) also showed two bands of similar relative intensity to those present in normal muscle.

Tropomyosin isolated by the standard procedure from rabbit cardiac muscle migrated as a single band of similar mobility to the faster and major component present in rabbit skeletal-muscle tropomyosin under all conditions in which the latter protein migrated as two bands (Plates 1a and 2b). A single band was also obtained with tropomyosin isolated from whole adductor muscle of the mollusc *Pecten maximus*. In

this case the band migrated more slowly than both bands observed in the tropomyosins from mammalian skeletal muscles. This difference in mobility is probably related to the differences in amino acid composition of tropomyosin from *Pecten maximus* compared with the tropomyosins from mammalian muscle, which were all very similar in composition (Table 2).

Although the mammalian skeletal-muscle tropomyosins all migrated on electrophoresis in sodium dodecyl sulphate as two bands of similar mobility, the ratios of the staining intensities of the bands differed between species. The ratios were characteristic of the species and constant in different preparations of tropomyosin from the same muscle (Table 3). When the specific radioactivities of the $[2-^{14}\text{C}]$ -carboxymethylated tropomyosins were determined after electrophoresis, the slower (β) component was shown to contain twice the cysteine content of the α component in all the tropomyosins isolated from skeletal muscle.

Discussion

The results presented above imply that the tropomyosin present in skeletal muscle contains at least two subunits which differ slightly in amino acid composition. We propose that the subunit present in the greatest amount in skeletal muscle and possessing one cysteine residue per molecule should be referred to as the α polypeptide chain and the minor subunit present containing two cysteine residues, as the β chain. Although from the isoelectric-focusing experiments it is apparent that the α and β fractions of tropomyosin obtained by chromatography each contain a minor component, it is not clear from the present study whether these minor components are additional polymorphic forms of the tropomyosin subunit. The amino acid sequence studies of Hodges *et al.* (1972) on the subunit of tropomyosin from rabbit skeletal muscle have indicated a minimum of four different polypeptide chains in standard tropomyosin preparations from rabbit skeletal muscle. Consequently it might be expected that under conditions of high resolution such as are obtainable with isoelectric focusing, tropomyosin would separate into a number of distinct components.

Provisionally we suggest that the major and minor components of the α fraction obtained by chromatography on CM-cellulose be referred to as α^1 and α^2 chains respectively and likewise for the components of the β fraction. Thus for precise description, the tropomyosin dimer should be referred to, for example, as the α_1^1 -tropomyosin or β_1^2 -tropomyosin, etc. Insofar as the terms tropomyosin A and tropomyosin B have been used to distinguish the paramyosin of molluscan adductor from the tropomyosin isolated from striated skeletal muscle, our proposals may

initially be considered to complicate the nomenclature. It should be pointed out, however, that Bailey (1957) and Laki (1957) originally considered paramyosin to be a form of tropomyosin and used the suffixes A and B to distinguish the two proteins. This convention has now almost fallen out of favour as it has been shown that paramyosin and tropomyosin, although somewhat similar in composition, are fundamentally different in function and structure. We consider the name tropomyosin should be reserved for the protein associated with actin in striated muscle, which is essential for the full activity of the troponin complex and which has the function of extending the effect of the periodically localized inhibitory-protein component of the troponin complex to neighbouring actin monomers (Perry *et al.*, 1972).

The evidence implies that the two main subunits of tropomyosin are under different genetic control and are not the consequence of proteolytic degradation of a single unique subunit during the preparative procedures. The differences observed on sequence analysis of tropomyosin (Hodges & Smillie, 1972*a,b*) are not readily explained on the basis that all sequences present are derived from an original unique sequence by partial proteolysis. Also the two-banded appearance of tropomyosin in the presence of sodium dodecyl sulphate is apparent on polyacrylamide-gel electrophoresis of freshly prepared whole myofibrils from skeletal muscle in which the possibilities of proteolysis by endogenous cathepsins are much less. Moreover, the two-band pattern is characteristic of the muscle type and in a given muscle the relative amounts of the subunits represented by the two bands is remarkably constant. The possibility that the minor α^2 and β^2 components arise by proteolytic degradation owing to endogenous cathepsins cannot be excluded at present.

Electrophoresis in sodium dodecyl sulphate suggests that the α and β chains differ in molecular weight. Some caution is necessary in drawing conclusions from these observations in view of the marked variation in the differences in molecular weight observed with different buffer systems and particularly in the presence of urea (cf. Tung & Knight, 1972).

Like myosin the precise form of tropomyosin present depends on the muscle type. In cardiac muscle the tropomyosin consists only of α chains with one cysteine residue, whereas the protein from mixed skeletal muscle contains 20–25% of the β chains containing two cysteine residues. Although it would be of interest to determine whether the α and β types of tropomyosin are produced by different muscle cell types, the present study does not permit a definite conclusion to be drawn on this point.

The case for the proposition that the α and β tropomyosins are polymorphic forms of the protein rests strongly on the fact that preparations consisting of only α subunits or only β subunits show similar

biological activity to each other and to the original preparation of tropomyosin from which they were isolated. Although the present studies indicate that tropomyosin dimers, α_2 and β_2 , are biologically active they do not indicate whether the $\alpha\beta$ dimer exists in preparations of tropomyosin from skeletal muscle and possesses the same biological activity. The existence of α_2 and β_2 forms of tropomyosin also poses the question as to whether in the I filament of the myofibril the tropomyosin filaments consist of one type of tropomyosin or whether a single filament of tropomyosin can be made of α_2 , β_2 and $\alpha\beta$ forms of tropomyosin.

The studies on the effect of tropomyosin on the actomyosin ATPase system indicate that all the biological properties associated with tropomyosin preparations are due to tropomyosin itself and not to uncharacterized impurities in the standard preparations. The studies involving thiol reagents, papain digestion and heat denaturation suggest that different parts of the molecule from those involved in its action with the troponin complex appear to be required for the effect on the Ca^{2+} -stimulated ATPase of actomyosin.

The precise role of tropomyosin in the regulation of the ATPase activity in the contraction-relaxation cycle in muscle remains obscure. Nevertheless it is clear that in some way it is involved in the interaction of myosin and the troponin complex with actin. The fact that two main forms of tropomyosin exist in skeletal muscle, which consists largely of fast fibre types whereas only one is present in cardiac muscle, implies some genetic response of tropomyosin synthesis to muscle function. It seems probable that the α and β forms of tropomyosin are polymorphic forms similar to the isoenzymes of creatine kinase and lactate dehydrogenase, the content of which in a given muscle reflects muscle type and function.

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