THE NATIVE SUBUNIT PATTERN OF TROPOMYOSIN

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1. Introduction

Tropomyosin is a rod-shaped protein with a molecular weight of 66 000–68 000 and is composed of two parallel, α-helical polypeptide chains [1–3]. It aggregates in vivo in head-to-tail fashion and lies in the grooves of the double-stranded F-actin filaments [4]. Each chain has a regular series of hydrophobic amino acid residues which are believed to interact with corresponding hydrophobes on the opposing chain, stabilizing the coiled-coil structure [5]. Analysis of the subunits on sodium dodecyl sulfate (SDS) polyacrylamide gel has revealed that there are two types – designated as α (the 34 000 dalton species) and β (the 36 000 dalton chain) – with the ratio of α to β varying with the type of muscle and species [6]. The nature of the assembly of the two subunit chains is unclear. It has been shown that $\alpha\alpha$ and $\beta\beta$ tropomyosins formed in vitro possessed biological activity equivalent to unfractionated tropomyosin [6]. However, hydroxylapatite chromatography separated tropomyosin into $\alpha\alpha$ and $\alpha\beta$, or single-band and double-band tropomyosins respectively, based on SDS polyacrylamide gels [7,8]. Subsequent rechromatography of the $\alpha\beta$ fraction failed to produce further separation into α and β components. Thus, whether the subunits are assembled as $\alpha\alpha$ and $\beta\beta$, $\alpha\alpha$ and $\alpha\beta$, or $\alpha\alpha$, $\alpha\beta$, and $\beta\beta$ dimers remains to be determined.

We have used the techniques of Stewart [9], Johnson and Smillie [10], and Lehrer [11] to form disulfide bonds between the opposing sulfhydryls on each chain of tropomyosin in order to define the subunit pairing in skeletal- and smooth-muscle tropomyosins. The results indicate that adult skeletal-muscle tropomyosin exists as $\alpha\alpha$ and $\alpha\beta$ while chicken-gizzard smooth-muscle tropomyosin contains $\alpha\alpha$ and $\beta\beta$ dimers.

2. Materials and methods

Tropomyosins from rabbit, pig and beef skeletal-muscle were prepared according to the method of Greaser and Gergely [12]. Single- and double-band fractions of tropomyosin were prepared by hydroxyapatite chromatography [7,8]. Chicken-gizzard smooth-muscle tropomyosin was a gift from Dr Robert Bremel. All samples were dialyzed against 1 M NaCl, 50 mM Tris–HCl, pH 8.5. Oxidation was performed by mechanically shaking 1 mg/ml solutions exposed to air for 36–48 h at 4°C. Samples were then dialyzed against 1% SDS, 10 mM phosphate, pH 7.0. To see the subunit compositions of each tropomyosin, SDS disc-gel electrophoresis [13] using 10% acrylamide was performed with dithiothreitol (DTT) added to reduce disulfides. The oxidized species were electrophoresed in the same manner in the absence of DTT. Gels were stained with Coomassie Brilliant Blue. To determine the subunit composition of the oxidized tropomyosins, two samples of each oxidized tropomyosin were electrophoresed. One sample of each species was stained and the other left unstained. Slices corresponding to each oxidized band were cut and then soaked overnight in 1 M DTT to reduce the disulfides. Each slice was then placed atop another SDS gel, electrophoresed, and stained.

3. Results

Figure 1 shows the subunit composition of the

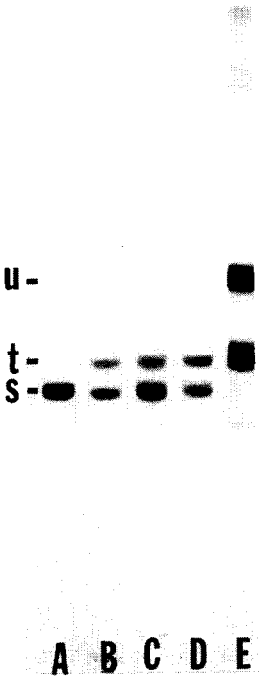

Fig.1. SDS polyacrylamide disc-gel electrophoresis of skeletal-muscle (A–D) and smooth-muscle (E) tropomyosins under sulfhydryl-reducing conditions. (A) Single-band rabbit tropomyosin, (B) Double-band rabbit tropomyosin, (C) Pig tropomyosin, (D) Beef tropomyosin, (E) Chicken-gizzard tropomyosin. Each sample included 10 μl of 1 M DTT. Position S corresponds to the α chain in gels A through D, position T corresponds to the β chain of skeletal-muscle tropomyosins (gels B–D) and the α chains of chicken-gizzard tropomyosin (gel E), and position U corresponds to the β chain of chicken-gizzard tropomyosin (gel E). Migration is from top to bottom.

various tropomyosins studied. Tropomyosin from skeletal-muscle of rabbit, pig, and beef gave either one or two bands when electrophoresed under sulfhydryl-reducing conditions. The α bands (position S, fig.1) of the tropomyosins from each species had nearly identical mobilities, as did the β bands (position T). The single-band αα tropomyosin (fig.1A) isolated by hydroxyapatite chromatography co-migrates with the α chains of the double-band fractions (fig.1B–D). The chicken-gizzard smooth-muscle α chain has a mobility similar to that of the β chain of skeletal-muscle while the smooth-muscle β chain is substantially heavier than skeletal-muscle β (fig.1E), a finding consistent with that of Cummins and Perry [6].

Oxidation of the αα tropomyosin yields a single band with molecular weight of about 65 000 on gels run in the absence of disulfide reducers (fig.2A, position v). However, oxidation of the αβ fraction produced two bands under the same conditions (fig.2B–D). The more mobile of the two bands co-migrates with the αα oxidized band. Two majors bands appear with oxidized gizzard tropomyosin (fig.2E), the more mobile (position y) migrating farther than the αα band of skeletal tropomyosin while the more retarded (position z) is substantially slower than any of the skeletal tropomyosin bands.

The results from the separation of oxidized tropomyosins and subsequent reduction are shown in fig.3. Reduction of the material from band v (fig.2A) resulted in the appearance of a single band at the α

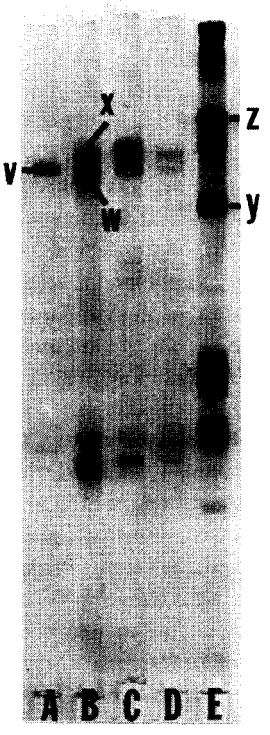

Fig.2. Electrophoresis of oxidized tropomyosins. (A) Single-band rabbit tropomyosin, (B) Double-band rabbit tropomyosin, (C) Pig tropomyosin, (D) Beef tropomyosin, (E) Chicken-gizzard tropomyosin.

Fig.3. Re-electrophoresis of oxidized tropomyosins. Each oxidized tropomyosin was electrophoresed and slices of unstained gels were removed which corresponded to the oxidized band regions shown in fig.2. The gel slices were submerged in 1 M DTT overnight and subsequently placed on top of another gel for a second electrophoretic run. (A) Single-band rabbit tropomyosin, band v of fig.2A, (B) Double-band rabbit tropomyosin, band w of fig.2B, (C) Double-band rabbit tropomyosin, band x of fig.2B, (D) Chicken-gizzard tropomyosin, band y of fig.2E, (E) Chicken-gizzard tropomyosin, band z of fig.2E.

chain migration position (fig.3A). A similar band (fig.3B) was obtained from the band at position w (fig.2B) after reduction. The position x band (fig.2B) yielded two bands (fig.3C) which corresponded to a mixture of the α and β chains. Figure 3 shows the results for the two bands from rabbit tropomyosin, but similar patterns were obtained from reduction of pig and beef tropomyosins as well.

Figure 3 also gives the gel pattern of the two major bands in the dimer molecular weight range of oxidized chicken-gizzard tropomyosin. The position y band (fig.2E) gave a single band after reduction, and its migration rate corresponded to that of the smooth-muscle α chain (fig.3D). The band at position z (fig.2E) had a similar migration position as the smooth-muscle β chain following sulfhydryl reduction (fig.3E). The small band between positions y and z in fig.2E gave no bands upon reduction and re-electrophoresis. The possibility remains that it might be an $\alpha\beta$ dimer, but the amount of material in this band is clearly much less than that found in the other two dimer bands. The higher molecular weight bands near the top of fig.2E presumably arise from formation of some intermolecular bonds (with a resulting size in the tetramer and larger range), and were thus not examined further.

4. Discussion

The present study clearly demonstrates that in adult skeletal-muscle of the species examined tropomyosin exists as $\alpha\alpha$ and $\alpha\beta$ dimers. No $\beta\beta$ could be detected. Amphlett and coworkers [14] have suggested that $\beta\beta$ tropomyosin is formed in fetal skeletal-muscle based on its high β:α ratio. The rate of α tropomyosin synthesis after birth exceeds that of β tropomyosin and $\beta\beta$ probably disappears due to muscle-protein turnover. In smooth-muscle, however, $\alpha\alpha$ and $\beta\beta$ were found to be the predominant subunit composition of the native molecules.

The existence of an $\alpha\beta$ tropomyosin raises interesting questions about the synthesis and assembly of tropomyosin. If only $\alpha\alpha$ and $\beta\beta$ tropomyosins were present in skeletal-muscle, then one could hypothesize that α chains would be synthesized in one type of fiber (white) and β chains in another (red). This pattern would account for the higher proportion of β chains red-muscle tropomyosin compared to white [6]. However, the fact that an $\alpha\beta$ tropomyosin is naturally formed means that the genes for both α and β tropomyosins are expressed in the same cell. One might expect then that $\alpha\alpha$, $\alpha\beta$ and $\beta\beta$ tropomyosins would then be produced. The apparent absence of a $\beta\beta$ tropomyosin suggests that the α and β chain synthesis may be tightly coupled to prevent the formation of a pool of free β chains.

In contrast to skeletal muscle, tropomyosin from the smooth-muscle examined exists as $\alpha\alpha$ and $\beta\beta$. The apparent of $\alpha\beta$ tropomyosin in smooth-muscle may indicate that $\alpha\beta$ cannot be assembled in the cell or

that there are different populations of smooth-muscle cells. The functional significance of the different tropomyosin types in both smooth- and skeletal-muscles remains to be determined.

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