TROPOMYOSIN FROM BOVINE BRAIN CONTAINS TWO POLYPEPTIDE CHAINS OF SLIGHTLY DIFFERENT MOLECULAR WEIGHTS

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1. Introduction

Skeletal muscle tropomyosin is a highly α-helical rod-shaped molecule which lies head-to-tail in the two grooves of the actin helix of the muscle thin filaments, and is an essential element of the Ca^{2+}-regulated control mechanism (reviewed [1]). Tropomyosin from rabbit skeletal muscle is a dimer, and on dissociation in the presence of SDS yields two polypeptide chains mol. wt 37 000, 33 000. Tropomyosins from different muscle tissues show considerable heterogeneity; however two types of polypeptide chains are always found which differ slightly in molecular weight or in isoelectric point [2,3].

Tropomyosins from non-muscle tissues appear to be composed of polypeptide chains of lower molecular weight (about 30 000) than their muscle counterparts [4–6]. However they are closely related to muscle tropomyosins: they form typical paracrystals [4,6], show similar amino acid compositions [4,5] and related chymotryptic fingerprints [6].

There may be only a single tropomyosin polypeptide chain in non-muscle cells [6]. We show here that bovine brain tropomyosin can be dissociated into two chains of slightly different molecular weights and, as has been shown for rabbit skeletal muscle [7,8], the polypeptide chains can be crosslinked by air oxidation of cysteine residues to form covalently-linked dimers.

2. Materials and methods

2.1. *Purification of tropomyosin*

Bovine brain tropomyosin was prepared as in [5]. The preparation involves making a dried ethanol/ether powder, after homogenisation of the washed tissue in ethanol. After overnight extraction with 1 M KCl buffer, the extract is boiled and the tropomyosin collected from the clarified supernatant by isoelectric precipitation, at pH 4.1. Ammonium sulphate precipitation between 40% and 53% saturation was used as a final step [5]. We added a step where a 47% ammonium sulphate pellet was back-extracted with 37% ammonium sulphate at 4°C. Paracrystals were prepared as in [4].

2.2. *Air oxidation*

Air oxidation of tropomyosin was performed as described [7], by stirring 0.7 mg/ml solution of tropomyosin in 1 N NaCl, 25 mM $CuCl_2$, 25 mM sodium borate (pH 9.3) at 20°C for 90 min. The product was characterized by SDS–polyacrylamide gel electrophoresis, avoiding mercaptoethanol in the sample buffer.

2.3. *Polyacrylamide gel electrophoresis*

Sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis was performed as described [9]. Urea/SDS gel electrophoresis was a modification of the method [10], and was identical to normal SDS–gel electrophoresis except that the running gel contained 6 M urea and the stacking gel and running buffer contained 3 M urea. An extract of rabbit skeletal muscle was used for molecular weight standards in gel electrophoresis.

3. Results and discussion

Bovine brain tropomyosin, purified essentially as

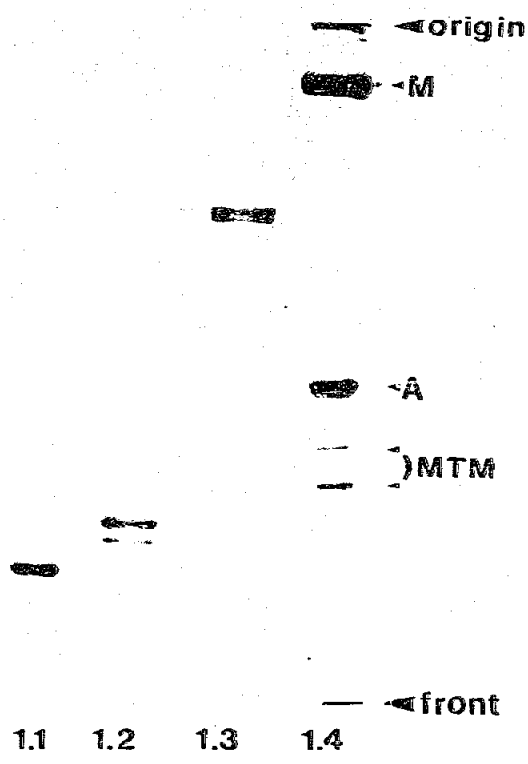

Fig.1. 10% SDS–polyacrylamide gel. (1.1) Carbonic anhydrase molecular weight marker (29 000). (1.2) Bovine brain tropomyosin prepared under reducing conditions. (1.3) Bovine brain tropomyosin after air oxidation and run in the absence of reducing agent. (1.4) Extract of rabbit leg muscle. (M) myosin; (A) actin; (MTM) muscle tropomyosin.

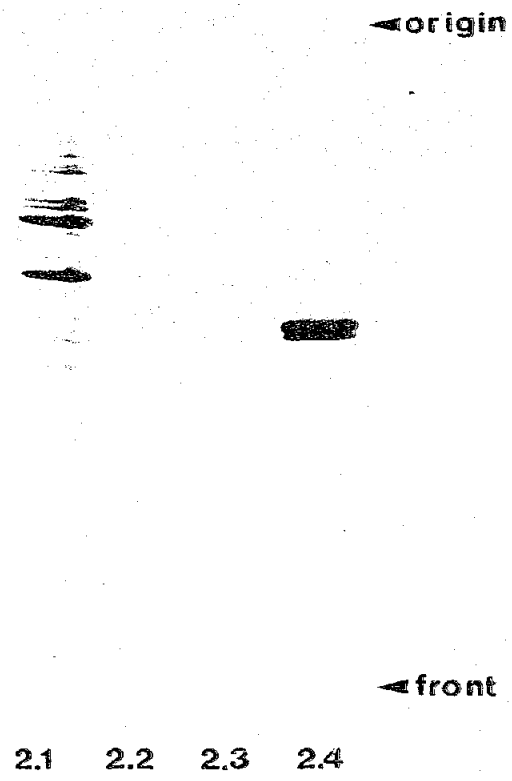

Fig.2. 15% SDS–polyacrylamide gel; preparation of bovine brain tropomyosin. (2.1) Extract from ethanol/ether powder. (2.2) Extract after boiling and removal of the precipitate. (2.3.) Precipitate obtained by isoelectric precipitation. (2.4) Purified tropomyosin.

in [5], gives rise to a closely-spaced doublet band of approx. mol. wt 30 000 (fig.1) on gel electrophoresis using the discontinuous SDS–Tris–HCl polyacrylamide gel system [9]. The report [6], indicating only a single polypeptide chain for non-muscle tropomyosin, was based on SDS electrophoresis in the continuous SDS polyacrylamide phosphate buffer system [11]. This gel system is known to show a lower resolution than the discontinuous SDS–Tris–HCl system [11], and therefore the difference in mobility was most likely too small to be detected. The yield of pure tropomyosin was about 0.5 mg/g dried powder, which represents about 0.1% total brain protein. The doublet band of tropomyosin can be seen on SDS–gels throughout the purification procedure (fig.2), suggesting that it did not arise from a single protein species by limited proteolytic degradation. A protein of approx. mol. wt 35 000 on SDS–gels was a minor contaminant of the purified protein. This contaminant was also found [6] and suggested to be smooth muscle tropomyosin from contaminating blood vessels, as it had a very similar chymotryptic map to calf smooth muscle tropomyosin.

The protein isolated has the characteristics of muscle and non-muscle tropomyosin. The isolation relies on the remarkable stability of tropomyosin to organic solvents and heat; when these properties are used in conjunction with isoelectric precipitation at pH 4.1, the major protein species left is tropomyosin (fig.2). The purified protein is able to form paracrystals in the presence of Ca^{2+}, a property of all tropomyosins [4–6]. Rabbit skeletal muscle tropomyosin is known

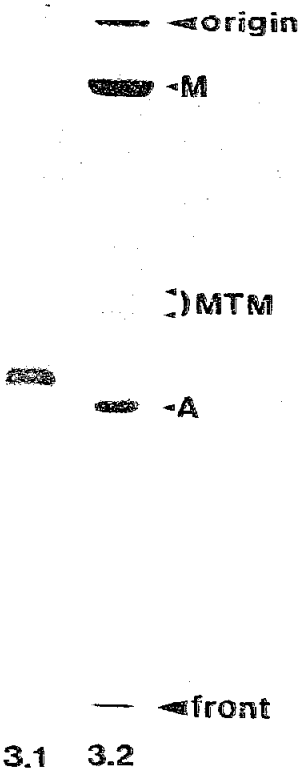

Fig.3. 10% Urea/SDS–polyacrylamide gel. (3.1) Bovine brain tropomyosin. (3.2) Extract of rabbit leg muscle. Abbreviations as in fig.1 legend.

to run with an anomalously high apparent molecular weight on urea/SDS gels ([10] and fig.3); similarly the bovine brain tropomyosin runs as a closely-spaced doublet with an approx. app. mol. wt 45 000 on urea/SDS gels (fig.3). This observation strongly suggests that both bands are tropomyosin.

Rabbit skeletal muscle tropomyosin contains one or two cysteine residues per polypeptide chain [2], which can be air oxidised to form an intramolecular covalent bond between the cysteine residues of the two chains [7,8]. These cysteine residues must therefore lie adjacent to each other in the native molecule and this implies that the chains are associated in register [7,8]. From its amino acid composition, platelet tropomyosin contains one or two cysteine residues per chain [4]. Brain tropomyosin must contain at least one residue per chain as air oxidation yields exclusively covalently-linked dimers (fig.1). On SDS–gels the dimers run as two bands (fig.1): since at least two dimers of slightly different molecular weights are formed one or both of the bands must represent molecules made from a single subunit type. Since no higher molecular weight molecules are observed, we propose, at least in the case of molecules containing two identical chains, that the cysteine residues which are crosslinked lie adjacent to one another and so the chains appear to be assembled in register.

Preliminary experiments have been carried out to determine whether other non-muscle cells also contain two tropomyosin polypeptide chains. Extracts were made from acetone powders of bovine thymus and SV40 virus-transformed mouse 3T3 tissue-culture cells, boiled and the soluble material run on SDS–gels. In both extracts, which are much enriched in tropomyosin compared to a crude cell extract, a doublet band was seen with precisely the same mobility as the bovine brain tropomyosin doublet band.

It appears that, in all tissues so far examined, there are at least two tropomyosin polypeptide chains. Similarly, two species of actin, differing in isoelectric point, have been found in non-muscle cells [10,12,13]. In none of these cases has the physiological significance of the two chains been understood.

Acknowledgements

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