Is There Any Difference Between Actins from Intact and Denervated Muscles?

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Abstract. Various techniques have been used (UV absorbance and fluorescence spectroscopy, polarised UV fluorescence microscopy, viscosimetry, electrophoresis and isoelectric focusing, superprecipitation, etc.) to establish definitely whether or not actin can be modified by denervation. As suggested by several parameters, denervation interferes with the structure of actin by producing local conformation changes in the region of aromatic residues near the sites responsible for actin-actin and actin-myosin interactions.

Key words: Conformational changes of actin — Effect of denervation on actin

Introduction

It is well known that the functional properties of skeletal muscles depend on the type of innervation, hormonal and external factors, and some pathological states of the muscles. This is due to changes occurring in contractile muscle proteins. A number of data concerning this subject are available in respect to myosin, whereas considerably less information is available on actin and proteins of the troponin-tropomyosin complex. This is explained essentially by the widely spread viewpoint that the structure of actin molecule is conservative (Elzinga and Collins 1973; Swezey and Somero 1982). Meanwhile, evidence has recently been obtained concerning the occurrence of actin isoforms (Whalen et al. 1976; Vandekerckhove and Weber 1978; Kohama 1980); this suggests that modified actin gets probably synthesized in muscle tissue under various physiological or pathological states. A proposal has also been made that different actin forms are related to different cell functions (Woodroofe and Lemanski 1981). Also, molecular evolution of muscle-specific actins has been suggested (Vandekerckhove and Weber 1978; Vandekerckhove et al. 1983). The present paper gives a survey

of our own and other studies on actin properties after denervation and under some pathological conditions. Our studies showed changes in specific properties of actin isolated from denervated skeletal rabbit muscles (in solution and fibres), thus confirming the possibility of its modification.

Materials and Methods

Protein preparation. Actin of white intact and denervated skeletal rabbit muscles (*extensor digitorum longus, gastrocnemius, biceps femoris, vastus lateralis*) was prepared from acetone powder (Straub 1942) and purified by two cycles of polymerization-depolymerization. The removal of free nucleotide from actin was made on a Dowex 1 × 8 column pretreated according to Estes and Moos (1969).

The muscles of one hind leg of a rabbit were denervated by the method described previously (Kalamkarova et al. 1976).

Myosin from intact skeletal muscles was prepared according to Szent-Györgyi (1947) with additional removal of actomyosin.

Protein concentration was determined with the biuret method.

Determination of actin activated myosin ATPase. Mg^{2+}-ATPase of synthetic actomyosin (myosin : actin weight ratio 4 : 1) was estimated from the yield of inorganic phosphate (Fiske and Subbarrow 1925) in a mixture containing 0.15 mol . l^{-1} KCl, 20 mmol . l^{-1} Tris-HCl, pH 7.2, 1 mmol . l^{-1} $MgCl_2$, 2.5 mmol . l^{-1} ATP and 0.1 mg protein in a total volume of 2 ml.

Measurements of UV absorption, UV fluorescence and polarised UV fluorescence. UV absorption spectra of actin solutions were recorded on a Specord-UV-spectrophotometer over a range between 240—300 nm. Tryptophan fluorescence spectra of actin solutions were studied by the method of Strankfeld (1963). Measurements of polarised UV fluorescence of muscle fibres were made by the method of Borovikov et al. (1983) on a polarisation microfluorimeter (Ioffe et al. 1974). The degree of fluorescence polarisation (P) was determined parallel ($P_\parallel$) and perpendicular ($P_\perp$) to the plane of the exciting light.

Viscosity measurements. Viscosity of protein solutions was determined with an Ostwald viscometer using a capillary with a diameter of 1 mm, and outflow time of about 11 s at 20 °C (solvent). The viscometer used had a capacity of 2 ml.

Flow birefringence measurements have been described previously (Kalamkarova and Mugeev 1957).

Superprecipitation of actomyosin. The superprecipitation of actomyosin suspension was studied by the turbidimetric method at a maximum transmission of 530 nm in a 2 cm thick glass cuvette at room temperature and under conditions as described earlier (Kofman et al. 1981). Following parameters were measured: E_{max} — maximum absorption increase; E_0 — initial absorption; $\Delta E = E_{max} - E_0$ — the value of superprecipitation; $t_{1/2}$ — the time for the half-maximal absorption increase.

Sodium dodecyl sulfate ($NaDodSO_4$)-polyacrylamide gel electrophoresis and isoelectric focusing. The standard technique of Davis (1964) was applied using 7 % polyacrylamide gel. Isoelectric focusing of actin was performed according to Whalen et al. (1976) and Swezey and Somero (1982) in 5 % polyacrylamide gel containing 1 % of ampholines (pH 4—6).

Aminoacid analysis. Actin samples in ampules containing 6N HCl were subjected to acid hydrolysis under vacuum for 24 hours at 110 °C ± 1 °C. After drying, washing with 350 mmol . l^{-1} acetic buffer pH 2.2, and filtration, the actin samples were studied using an amino acid analyzer Hitachi KLA-3 (accuracy 3—5 %).

Myofibrils were prepared according to Perry and Zydowo (1959)

Results

SDS-electrophoresis and isoelectric focusing. Figure 1 shows the results of an electrophoretic study of myofibril preparations from intact and denervated

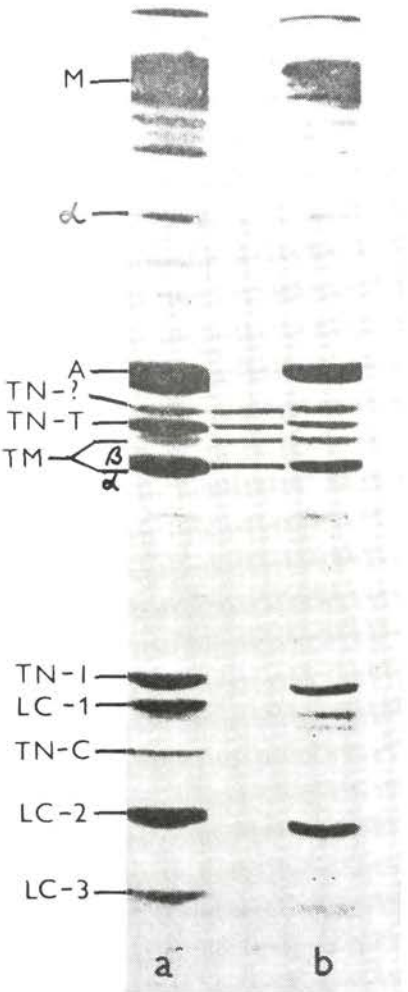

Fig. 2. Electrophoretic analysis of actin in the presence of sodium dodecylsulphate: *a* — intact actin, *b* — actin from denervated (14 days) muscles.

Fig. 1. Electrophoretic analysis of glycerinated fibres preparations: *a*) intact glycerinated fibres, *b*) denervated glycerinated fibres (14 days). M, heavy myosin chains; *α*,*α*-actinin; A, actin; TN-T, TN-I, TN-C, TN-?, troponin components; TM-*α*, TM-*β*, *α* and *β* tropomyosin; LC-1, LC-2, LC-3, light myosin chains.

actin, denervated actin showed a reduced viscosity. Minimum values were found 14—15 days after denervation. There was a temporal coincidence of minimum values of viscosity and UV fluorescence intensity for denervated actin. Viscosity changes were the same after purification of actin (Fig. 5). At this time (14 days after denervation) the minimal values of flow birefringence of denervated actin were observed (Fig. 6). As described previously (Strankfeld et al. 1975) the viscosity of actin increased extremely in the presence of a basic dye acridine orange at high concentrations of KCl and NaCl. After denervation this "super-viscosity" effects diminished significantly or could not be observed at all. The viscosity of synthetic actomyosin which contained denervated actin decreased as compared to the intact complex, to reach its minimum in two weeks. A partial reversibility of these changes was observed at later times.

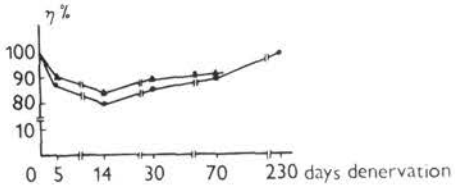

Fig. 5. Relative viscosity of F-actin from denervated muscles prior to purification (●) and following purification by polymerization-depolymerization cycles (▲). Protein concentration 1.5 mg/ml.

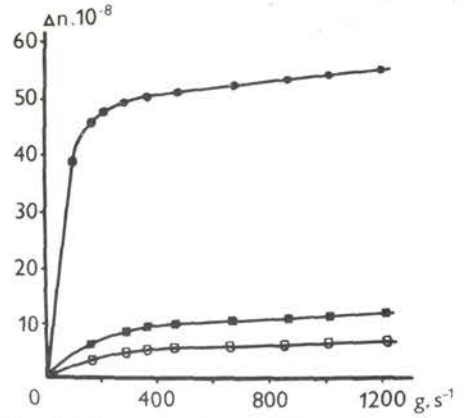

Fig. 6. Changes in flow birefringence of intact actin and actin from 14-days denervated rabbit skeletal muscles. (○) intact G-actin, (●) intact F-actin, (□) G-actin from denervated muscle, (■) F-actin from denervated muscle.

Measurements of actin activated myosin ATPase. Throughout the post-denervation interval (2—75 days) the capacity of denervated actin to activate myosin Mg^{2+}-ATPase did not change (intact actin + myosin — 0.48 ± 0.10 mmol $P \cdot mg^{-1} \cdot min^{-1}$; denervated actin + myosin — 0.46 ± 0.13 mmol $P \cdot mg^{-1} \cdot min^{-1}$); in other words, the effect did not differ from normal at some actin-myosin ratio.

Superprecipitation studies. Under our experimental conditions no clearing phase, associated with actomyosin dissociation, was actually observed in the samples studied. Curve 1 in Figure 7 illustrates typical superprecipitation of intact synthetic actomyosin. Interaction of denervated actin with intact myosin

significantly affected both the value and the rate of superprecipitation. As seen in Figure 7, ΔE fell to 75.4 ± 7.3 % ($p = 0.95$) with actin extracted on the 14th post-denervation day. With actin extracted on the 75th post-denervation day (Fig. 7, curve 3) ΔE somewhat restored ($\Delta E = 87.9 \pm 11.2$ %, $p = 0.95$) and approached its normal value. Calculations showed a 41 ± 13.6 % ($p = 0.95$) increase in $t_{1/2}$ for actomyosin containing the 14-day denervated actin; a value as low as 17.5 ± 11.9 % ($p = 0.95$) was found for actomyosin containing 75-day denervated actin. Since $t_{1/2}$ was the inverse value of superprecipitation velocity the latter was supposed to slow down at the introduction of denervated actin into the complex.

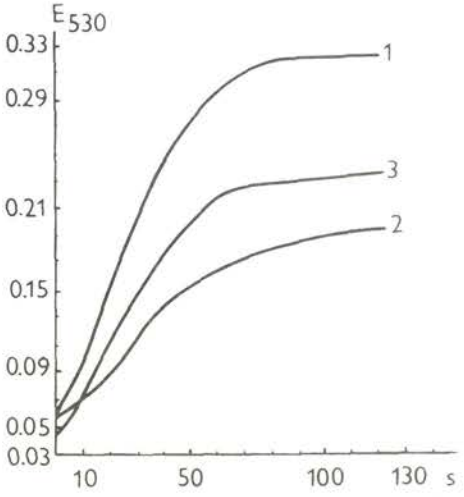

Fig. 7. Superprecipitation of synthetic actomyosin. 1 — intact actomyosin, 2 — intact myosin + F-actin following 14-days denervation, 3 — intact myosin + F-actin following 75-days denervation. Synthetic actomyosin was prepared by adding 2.5 parts of myosin on 1 part of actin (by weight). The final protein concentration in each test sample was 0.14 mg/ml.

Table 1. Polarization of tryptophan fluorescence of intact and denervated rabbit muscle fibres

Muscle fibres	$P_\perp$	P	$P_\perp/P$
		Glycerinated Fibres	
Intact	0.138 ± 0.003	0.375 ± 0.005	0.368 ± 0.010
Denervated (50 days)	0.167 ± 0.006	0.341 ± 0.008	0.500 ± 0.015
		Ghost Fibres	
Intact	0.222 ± 0.002	0.200 ± 0.001	1.110 ± 0.020
Denervated (14 days)	0.228 ± 0.002	0.189 ± 0.002	1.210 ± 0.025
Denervated (30 days)	0.230 ± 0.003	0.180 ± 0.002	1.280 ± 0.030
Denervated (50 days)	0.232 ± 0.003	0.176 ± 0.003	1.320 ± 0.040

No less than 15 fibres taken from 5 rabbits were tested in each experiment.

Measurements of amino acid contents. The amino acid analysis of intact and denervated actins showed that by the 14th day after denervation the contents of valine, histidine and tyrosine decreased, whereas those of leucine and lysine increased (Table 2). The contents of all these compounds nearly reached control levels by the 50th day.

Discussion

Data obtained using various methods showed that denervated actin differs from the intact one in several respects. A reduced viscosity as well as a decrease in flow birefringence of denervated F-actin suggest that denervated G-actin loses its ability to polymerize. Reduced asymmetry of actin aggregates in solution suggests alterations in structure-forming properties of actin to occur including change in G-F transition capacity. The polymer state of actin seems to contribute more to the contraction process than does the monomer state. The loss of actin ability to form polymer structures may thus be responsible for functional changes occurring in muscles after denervation.

The decreased viscosity of actomyosin containing denervated actin seems to indicate a reduced capacity for complex formation between denervated actin and intact myosin. Certainly, changes in the intrinsic viscosity of actin mentioned above should also be considered; however, they alone cannot account for this effect.

The main result of our superprecipitation experiments was that two weeks following denervation both the value and the velocity of superprecipitation of synthetic actomyosin containing denervated actin decreased; this is in full accordance with data on natural actomyosin (Samaha and Thies 1973). Since myosin with intact properties was used for reconstruction of the complex all the changes in superprecipitation observed were associated with alterations of actin rather than myosin. It can be assumed that the increase in $t_{1/2}$ is associated with deceleration of formation of superprecipitation centres. This is probably due to the loss of the ability of a portion of actin to form complexes with myosin involved in superprecipitation. The decrease in ΔE is due to a reduction in the number of superprecipitation centres formed. The superprecipitation changes can probably be explained by the existence in the above complex of actin with an altered structure-forming properties, probably as a result of conformational changes. It should be noted that in some pathological states of the heart muscle changes occur in actin sites responsible for polymerization and actin-myosin interaction (Karsanov and Jinchvelashvili 1981).

An increase in absorption at 260 nm is associated with the nucleotide component of denervated actin. As no special information is available on the content

of bound nucleotide in denervated actin it can be supposed that increase in the total amount of nucleotides in muscles is a result of an intensified synthesis of nucleic acids following denervation (Graff et al. 1965).

The above mentioned decreased intensity of UV fluorescence (of actin solution and actin in ghost fibres) and the changed polarised UV fluorescence (of actin in ghost fibres) indicate that denervation induces changes in actin structure in regions in which actin-actin and actin-myosin interactions take place (Borovikov et al. 1983). The analysis of polarised fluorescence showed different conformational changes in F-actin of denervated and intact muscles in response to the same reagents — ATP and phalloidin (Borovikov et al. 1984). Based on these data it was assumed that the N-terminal peptide involved in actin polymerization (Elzinga and Collins 1973) was changed during denervation.

At present, it is not easy to identify exactly the changes discovered above in the contents of some amino acids following denervation. However, these data agree with the concept concerning the role of some amino acids (histidine, lysine) in polymerization of actin (Lu and Szilagyi 1981) and its interaction with myosin (Martonosi and Gauvea 1961). It is worth noting that a decreased histidine content was observed in actin in a case of hereditary myopathy (Badaliyan et al. 1970).

Information about actin synthesis would be of great importance for the analysis of our data; however the available reports are very conflicting. Some authors could not show any changes in the rate of actin synthesis following denervation (Srivastava 1972). According to Kohama (1980) the incorporation of [^{3}H] labelled amino acids into actin is increased during the development of chicken muscle dystrophy.

On the basis of the given data it is difficult to establish exactly at what organizational level of the actin macromolecule the changes occur. It seems unlikely that during the process of atrophy it is the quarternary structure of this protein alone that changes. In actin of denervated muscles local conformational changes must occur resulting in disturbances in actin-actin and actin-myosin interactions. Conformational changes may be suggested to occur in the region of aromatic residues near to the sites responsible for actin polymerization (probably in the N-terminal peptide). Such local conformational changes in actin monomers are not important for activation of actomyosin ATPase.

Of interest is the fact that some parameters of denervated actin approach the norm at later time intervals after denervation. One of the possible reasons may be the development of nerve collaterals. However, even if they are formed they would evidently be inadequate to restore protein synthesis and to normalize the muscle function. It should be noted that reversible changes did not concern all the actin parameters: the flow birefringence of denervated actin was not restored (Kalamkarova et al. 1972). The synthesis of modified actin probably increases

during the atrophy development (Goldspink 1976), but the extractability of this actin may be lower than that of intact actin. Restoration of some of the parameters was observed at later time intervals following nerve section, so the portion of intact protein in solution increased. A luminescence study of actin in ghost fibres also showed that the optical properties of actin did not restore at later time intervals after denervation (Borovikov et al. 1983).

Our data do not allow to rule out the possibility that changes in protein conformation in vivo may also be associated with the activity of proteinases. Unfortunately, our understanding of this problem is rudimentary.

Similary as in myosin (Syrový 1976; Kalamkarova et al. 1976) the observed structural alterations in actin are also accompanied by functional changes of denervated muscles.

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