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YIELDS OF EASILY RELEASABLE MYOFILAMENTS AND MYOFIBRILLAR PROTEOLYSIS IN DENERVATED MUSCLE.

Brendan E. Murray[1] and William J. Reville[2]

[1]*Teagasc, The National Food Centre, Dunsinea, Castleknock, Dublin, Ireland and*
[2]*Department of Biochemistry, University College Cork, Cork, Ireland.*

Introduction

A small sub-population of myofilaments, termed easily releasable myofilaments (ERM) is readily released from the surface of myofibrils under relaxing conditions (i.e. 3mM MgATP in 0.1mKCl, 3mM EGTA, 1mM DTT, 20mM tris-maleate, pH7.0 – Low Salt Buffer [LSB]). It has been proposed that ERM are early intermediates in the turnover of myofibrils (1). If this hypothesis is correct it would follow that ERM yields would be increased in conditions where proteolysis of myofibrils is elevated, and in several instances this has been shown to be the case. Increased yields of ERM have been found in post-mortem bovine chicken and porcine muscle (2) and in Vitamin-E deficient muscle (3).

In many cases the detection of myofibrillar proteolysis is a rather subtle matter. The conventional method of examination of myofibrillar protein subunit compostion by polyacrylamide gel electrophoresis in the presence of sodium dodecylsulphate (SDS-PAGE), although quite sensitive, usually detects but a weak signal of proteolysis even when proteolysis is known to be elevated, e.g. as evidenced by SDS-PAGE examination of myofibrils during the post-mortem ageing of beef. However, if much of the activity during myofibrillar proteolysis takes place in the small ERM sub-population of myofilaments, then closer examination of the ERM fraction by SDS-PAGE should more easily detect evidence of proteolysis in conditions where proteolysis is enhanced.

Myofibrillar proteolysis is elevated in denervated muscle. In this present study ERM yields were measured in denervated rat skeletal muscle and the ERM fraction from denervated muscle was carefully examined by SDS-PAGE for evidence of proteolysis.

Objectives

To determine if ERM yields are elevated in denervated muscle and to examine ERM by SDS-PAGE for a strong signal of proteolysis.

Methodology

Myofibrils were prepared from the gastrocnemius muscle by the rigor method from groups of mature male Wistar rats (360-399g). Denervation was effected by the surgical removal of a 1 cm section of the sciatic nerve from the upper right hind limb. The muscles of the untouched contra-lateral limb served as the control samples. Because the

individual gastrocnemius muscles are small, 4-5 animals were used for each control/denervated sample pair in order to allow sufficient ERM protein to be prepared to calculate ERM yields. Data from ten such paired samples were statistically analysed using a paired t-test. The animals were denervated 14 days prior to sacrifice. ERM were prepared from myofibrils as previously described (2) and myofibrils and ERM were subjected to SDS-PAGE, with separation achieved on an 8-18% linear gradient slab gel.

Results & Discussion

Denervation for 14 days caused a marked decrease in the size of the gastrocnemius and the soleus muscles. Control gastrocnemius weighed 1846±107 mg, denervated gastronemius weighed 1110±161 mg, control soleus weighed 146±12 mg, and denervated soleus weighed 80±8 mg (means±1s n=4 animals).

ERM yields from control and denervated muscles are illustrated in Figure 1. ERM yield from denervated muscle, at 3.69±0.75% of total myofibrillar protein, was 56% greater than ERM yield from control muscle at 2.37±0.45% (means±1s, n=10, $P \leq 0.001$). Protein release measured in the absence of Mg-ATP i.e. in presence of LSB alone, was very low but the means here were also significantly different – 0.54±0.18% total myofibrillar protein in denervated muscle vs 0.38±0.17% in control muscle (means±1s, n=10, $P \leq 0.001$).

Figure 2 illustrates the myofibril and ERM protein subunit profiles as separated on SDS-PAGE from control and denervated gastrocnemius muscle. The main protein bands are readily identified as myosin, α-actinin, actin, thin myofilament regulatory proteins and thick myofilament associated proteins. Little difference is discernible between control and denervated myofibrils (lanes 2 and 3, control and denervated muscle respectively, on SDS-PAGE), despite the considerable wasting of the denervated muscle.

In consonance with the increased ERM yields from denervated muscle, the ERM protein subunit profiles on SDS-PAGE differ markedly from each other between control and denervated muscle (Figure 2, lanes 4 and 5 – control and denervated muscle respectively). An extensive ladder of bands running from approximately 33kDa to 14kDa was apparent in the denervated ERM fraction, but not in the control ERM fraction. There was also a noticeable decrease in the myosin to actin ratio in the ERM denervated fraction compared to control ERM.

The extensive ladder of lower molecular weight bands that appears in denervated ERM presumably reflects proteolytic breakdown products of higher molecular weight proteins. This indicates that myofibrillar proteolysis is concentrated in the ERM fraction, i.e. the loosely attached peripheral myofilaments of the myofibril, because these breakdown products are not at all obvious when the whole myofibril is examined. Since ERM protein constitutes only 2 to 4 percent of total myofibrillar protein, the apparent effects of proteolysis, if concentrated in this ERM fraction, would be massively diluted when the whole myofibril is examined by SDS-PAGE.

Other work in this laboratory has shown that ERM from post-mortem bovine muscle also display the progressive appearance, as ageing of the muscle proceeds, of protein subunit in the molecular weight range 22kDa to 45kDa, and these bands are much more readily apparent in ERM than in preparations of whole myofibrils examined by SDS-PAGE.

ERM in post-mortem muscle also show a decreased myosin to actin ratio compared to whole myofibrils, similar to that seen in denervated muscle, and the decreasing ratio is progressive with extent of post-mortem ageing. This is mostly likely explained by Z line weakening due to proteolysis leading to an easier release of thin myofilaments thereby enhancing the ratio of thin to thick myofilaments in ERM preparations.

Many investigations of myofibrillar changes in muscle in conditions of increased protein turnover or studies of the post-mortem ageing of meat have concentrated on whole muscle samples or on myofibril preparations (4, 5). Very few studies of changes in myofibrils under conditions of enhanced proteolysis have concentrated on ERM. Future studies in this area might more fruitfully concentrate on ERM where the signal of proteolysis is more easily detected.

Conclusions

ERM yields are significantly increased in rat skeletal muscle after 14 days of denervation compared to undenervated control muscle. ERM prepared from myofibrils from denervated muscle show clear evidence of proteolysis on examination by SDS-PAGE. Since this evidence is not readily apparent in whole myofibrils prepared from denervated muscle, it seems that the signal of myofibrillar proteolysis is much more readily detectable in the ERM fraction. This has significance for future studies of myofibrillar proteolysis in various conditions.

References

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Tables and Figures

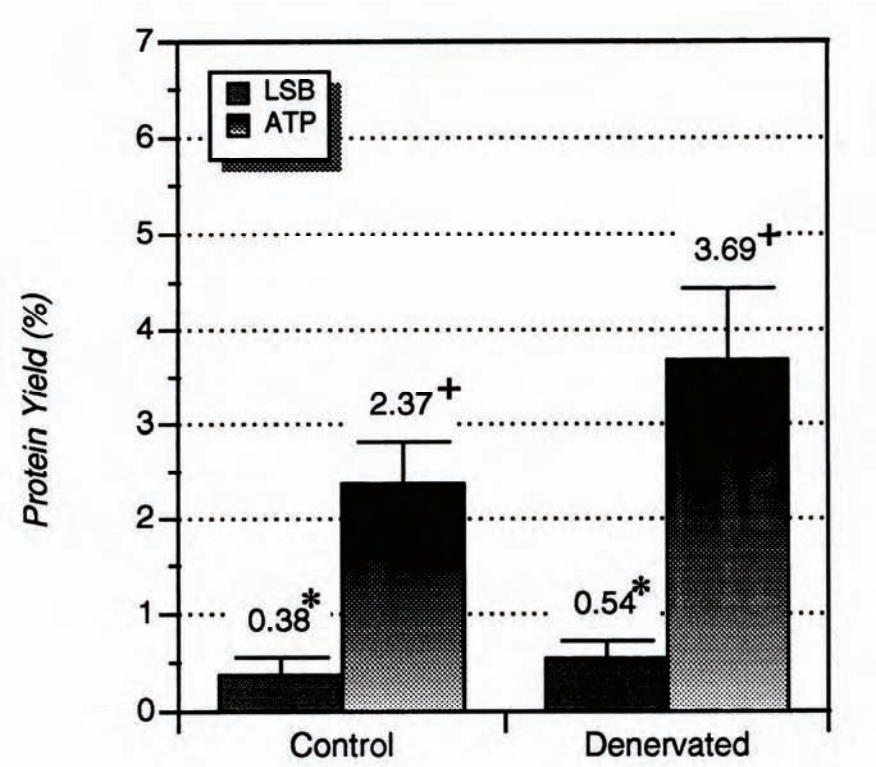

Figure 1. ERM yields (in presence of Mg-ATP and Low Salt Buffer [LSB]) and protein released from myofibrils in the absence of Mg-ATP (in presence of LSB alone) from rat gastrocnemius muscle after 14 days denervation and from control contra-lateral gastronemius muscle that was not denervated. Yield are expressed as percentage of total myofibrillar protein. Bars represent means±1s, n=10 paired preparations of control and denervated samples (4-5 animals were required for each control/denervated sample pair). Significant differences were observed between LSB control vs LBS denervated and between ERM (ATP) control vs ERM (ATP) denervated as assessed by paired t-test (P≤0.001).

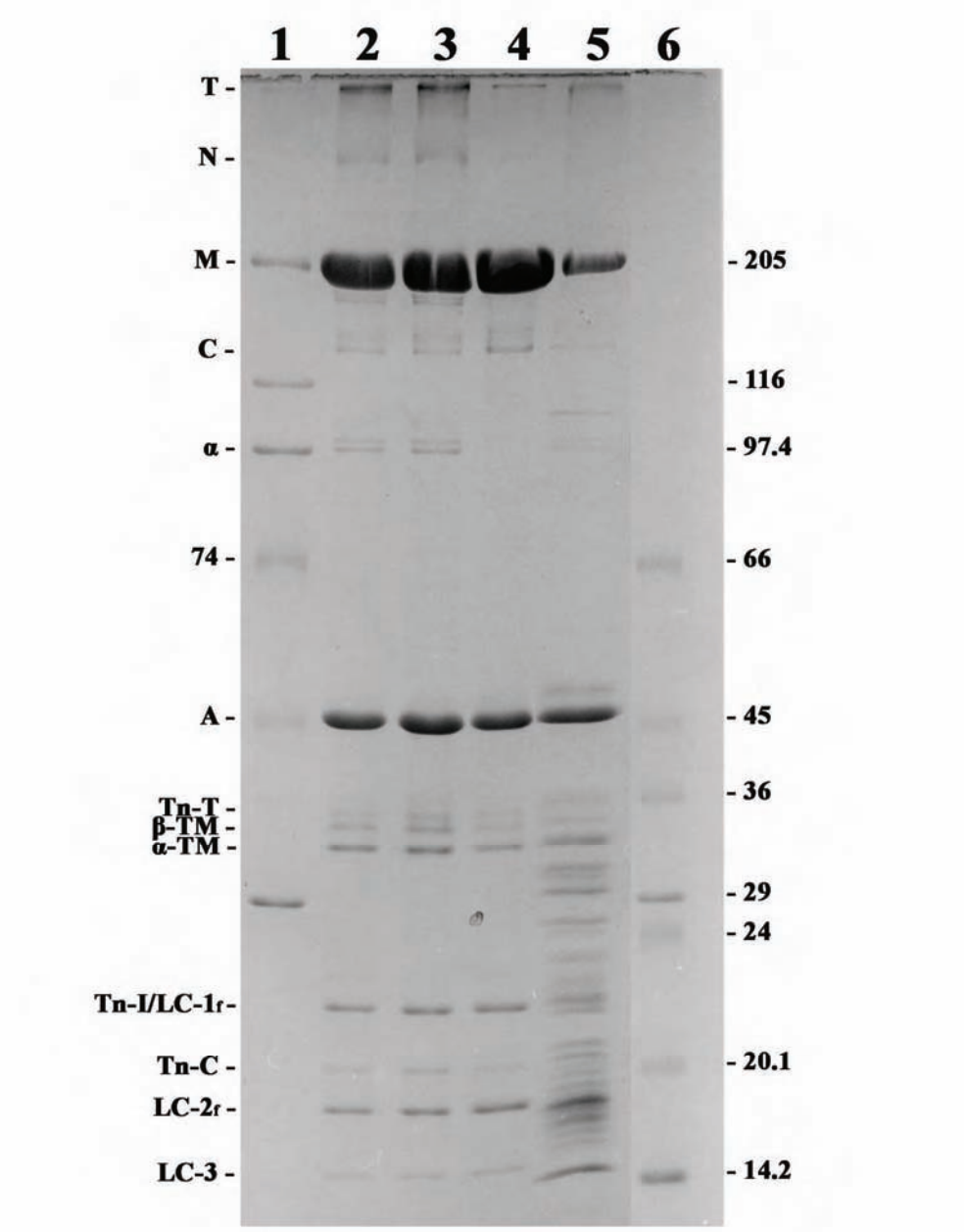

Figure 2. SDS-PAGE profiles of myofibrils and ERM from rat gastrocnemius muscle from control and 2 week denervated animals. Lanes 1 and 6 correspond to high and low range molecular weight markers respectively. Numerical values are quoted as kDa. Lanes 2 and 3 are myofibrils from control and denervated muscle respectively. Lanes 4 and 5 are ERM from control and denervated muscle respectively. 30μg total protein was applied per well and separation was achieved on an 8-18% linear gradient slab gel. T: titin; N: nebulin; M: myosin; C: c-protein; α: α-actinin, A: actin; TnT: troponin-T; TM: α, β-tropomyosin; LCl_f: fast isoform of myosin light chain-1; Tn-1: troponin-1; TnC: troponin C; $LC2_f$: fast isoform of myosin light chain-2; LC3: myosin light chain-3.