4-P16

Effect of Exogenous Addition of Gelsolin on Myofibrils Prepared from Bovine Muscle.

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Introduction

First discovered in macrophages (Yin and Strossel, 1979), gelsolin is now known to be present in mammals in both cytoplasmic and plasmic forms, as well as being a constituent of bovine skeletal muscle (Kwaitkowski et al., 1988 a, b). Regardless of source, the functions and biological properties of the protein are the same. Gelsolin is a calcium-dependent, actin-binding, nucleating and severing protein (Yin and Strossel, 1979, 1980; Yin et al, 1981; Kurth et al., 1983). It can bind up to 2 actin monomers (GA2) in the presence of Ca^{2+}, but the C-terminal actin binding site loses its ability to bind G-actin in the presence of EGTA (Forscher, 1989). Only the GA2 complex can serve to nucleate actin polymerization. Gelsolin can sever F-actin and cap the barbed end of the filament to prevent reannealing of the polymer. Ca^{2+} binding to the C-terminal half of gelsolin results in a conformational change which "opens" up the F actin binding site (Pope et al., 1997). Gelsolin can sever thin filaments in myofibrils (Fuatsu et al., 1990, 1993), although severing is much more efficient in the absence of thin filament associated proteins such as nebulin (Gonsior and Hinssen, 1995). The process of postmortem ageing of muscle results in degradation of thin filament associated proteins and release of a-actinin from the Z-lines (Goll et al., 1989). The binding and severing action of gelsolin under these conditions could result in loss of structural integrity and disassembly within the myofibril.

Objective

The aim of this research was to examine the binding and severing activities of gelsolin on myofibrils prepared from bovine muscle after various postmortem ageing times.

Methods and materials

Bovine blood and sternomandibularis muscle used in this study was collected at a local abattoir. Myofibrils were prepared from the muscle at death and again after 7 and 14 days storage of the muscle at 4°C. The myofibrils were prepared as described by Murray (1997), and gelsolin was purified according to the method of Kurokawa et al, (1990). Gelsolin additions to myofibrils were carried out in the presence of either 1 mM EGTA or 1 mM Ca^{2+}, at an estimated 1:20 molar ratio of gelsolin to actin (it was assumed that 1 mg myofibrils contains 250 µg of actin). After a 30 min incubation period, the myofibrils were washed twice to remove unbound gelsolin and excess Ca^{2+}.

For the fluorometric assays, the samples were incubated with monoclonal anti-gelsolin antibody (GS-2C4, available from Sigma Chem. Co.) in Tris Buffered Saline (TBS) for one hour followed by 3 rinses with TBS. They were then incubated with BODIPY labelled antimouse IgG (Sigma Chem. Co.). After washing to remove unbound antibody, the samples were placed in a Perkin Elmer Luminescence Spectrometer LS 50B and the fluorescent intensity was measured. Samples incubated with secondary antibody only were used as a blank.

Disassembly of myofibrils was determined by measuring the amount of myofilaments released in the presence of a relaxing buffer containing Mg-ATP plus EGTA, following a gentle shearing action (Murray, 1997). These myofilaments are termed the easily releasable myofilaments (ERM) (van der Westhuyzen et al., 1981). Myofibrils previously treated with gelsolin and Ca^{2+} were rinsed two times with low salt buffer (LSB) to remove unbound gelsolin and excess Ca^{2+} prior to ERM release. The ERM yield was measured as total protein released from the myofibrils upon shearing, expressed as a percentage of the initial myofibril protein. In order to pinpoint the final destination of gelsolin in the ERM fraction, these fractions were centrifuged at 100,00Oxg to separate the ERM pellets and supernatants.

Results and Discussion

Very little change in background fluorescence was observed in the control samples between day 0 and day 14 myofibrils. However there was a four fold increase in fluorescence intensity over the ageing period in samples treated with gelsolin and 1 mM Ca^{2+}. This fluorescence intensity represents gelsolin that has presumably formed caps on the severed actin filaments, and protein that has bound to the filaments without severing. Day 7 and day 14 myofibrils bound much more gelsolin than at death myofibrils, due to the increased availability of actin binding sites, presumably brought about by calpain digestion of nebulin during the first 7 days postmortem (Greaser and Fritz, 1995). Nebulin is a large thin filament associated protein which confers actin resistance to gelsolin, when present in intact myofibrils (Gonsior and Hinssen, 1995). Loss of a-actinin from the Z-line and loss of Z-line integrity also "loosens" the thin filaments anchor and allows for enhanced gelsolin accessibility to the thin filament. These effects are more pronounced after 14 days postmortem, which may be due to continuing loss of competing a-actinin (Hwan and Bandman, 1989).

Immunofluorescence microscopy of myofibrils at day 0, 7 and 14 showed a similar pattern of gelsolin binding. In the presence of 1 mM Ca^{2+}, gelsolin was visualized primarily along the Z-line region, and in increasing amounts as the muscle aged.

ERM yields in bovine muscle increase significantly between day 0 and day 14 postmortem, rising to about 5.5% of total myofibrillar protein by 14 days postmortem (Reville et al, 1994). However, myofibrils treated with gelsolin in 1 mM Ca^{2+}, show even greater ERM yields, reaching 5.5 % at day 7 and over 16% by day 14. There is a five fold increase in ERM yield in gelsolin and Ca^{2+} treated samples between day 0 and 14. The enhanced ERM yields obtained from myofibrils treated with Ca^{2+} and gelsolin reflect the increase in thin filament severing achieved through gelsolin binding.

Conclusions

Exogenous gelsolin bound to myofibrils, in the presence of calcium, during all stages of postmortem ageing of bovine skeletal muscle. Gelsolin binding increased as postmortem ageing proceeded. Easily releasable myofilaments (ERM) yields from these gelsolin bound myofibrils were enhanced, which indicates increased release of thin filaments due to gelsolin severing activity. These results demonstrate the potential importance of gelsolin intracellular activity postmortem in effecting many of the well characterized degradative effects in the myofibril.

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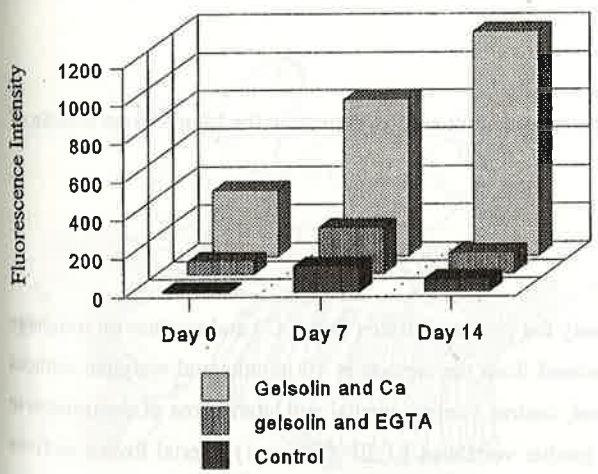

Figure 1: The level of gelsolin bound to myofibrils prepared from bovine muscle after different periods of ageing was measured as the level of fluorescence intensity (I_f) recorded for anti-gelsolin detection antibody, labelled with BODIPY. I_f was measured in solution, using a Perkin Elmer Luminescence Spectrometer, Model LS 50B.

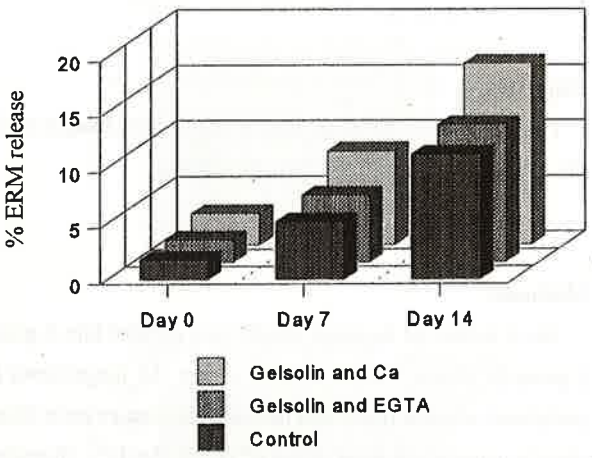

Figure 2: Effect of gelsolin on ERM release from myofibrils prepared from bovine muscle after various postmortem ageing times. ERM yields are expressed as percent ERM protein of total starting myofibrillar protein. Gelsolin was added at an approximate 1:20 molar ratio to actin, in the presence of either 1mM Ca or 1 mM EGTA.