SEQUENCE ANALYSIS OF THE 110 kDa MYOFIBRILLAR PROTEIN FRAGMENT

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INTRODUCTION

One of the most important criteria for consumer acceptability of meat is tenderness. The major factor affecting meat tenderness is ageing. Proteolytic degradation which occurs early post-mortem as part of the ageing process results in the production of protein fragments. The most noted of these is the 30 kDa fragment which has been shown to originate from troponin T. It has been demonstrated that the 30 kDa fragment (Negishi et al, 1996) and a second fragment, the 110 kDa fragment (O'Halloran et al, 1996), appear during ageing on SDS-PAGE of myofibrils from bovine muscle. They are therefore potentially useful in the prediction of meat tenderness. The 110 kDa fragment appears in much smaller quantities than the 30 kDa fragment. Its origin has been reported previously (Troy et al, 1997). However, the sequence analysis data is limited to 17 residues. With a synthetic portion of the fragment an enzyme-immunoassay type test could be developed for evaluating levels of the 110 kDa fragment as a predictor of meat quality. In this work we present data which confirms the original sequence reading published and extends it by ten residues. The origin of the 110 kDa fragment is also confirmed.

OBJECTIVES.

This investigation describes the isolation, origin and sequence analysis of the 110 kDa protein fragment.

METHODS

Preparation of samples

Sections of M. *Longissimus dorsi* were excised from Hereford cross heifers at 24 h post-mortem day and aged at 3 ± 10 °C for 2, 7 and 14 days. The muscle was trimmed of fat and connective tissue, and finely chopped. Meat samples are placed in capped tubes, frozen in liquid nitrogen and stored at -70 °C. Myofibrillar proteins were extracted from these samples using the method developed by Wang (1982). The freeze-dried myofibrillar proteins were concentrated further by dissolving them at a concentration of 8.66 mg/2 ml of 0.5 M Tris, pH 6.8, vortexing and mixing overnight. The samples were centrifuged at 8,720 x g for 5 min and 1.5 ml of the supernatant was freeze-dried. The residue was resuspended in approximately 150 µL of sample buffer for SDS-PAGE.

SDS-PAGE electrophoresis and electroblotting

SDS-PAGE was carried out according the method of Greaser et al (1983) using a 3.0% stacking gel and a 7.5% separating gel. The high range of SDS-PAGE protein standards (BIO RAD) were used as molecular weight markers. Separation was run over 4 hours at a constant voltage of 165V (Consort E831 power box). The resulting protein bands were electroblotted onto polyvinylidene difluoride (PVDF) membrane (ProBlott™ from Applied Biosystems) using the method published by Szewczyk et al. (1994). Transfer was achieved using 0.1 M CAPS (3-[cyclohexylamino]-1-propanesulfonic acid) buffer, pH 11, with 10% methanol and a constant power of 20V overnight (approximately 16 hours). PVDF blots were stained with Coomassie Blue dye and destained with 100% methanol followed by 50% methanol, washed in distilled water and dried in air.

Sequencing analysis

Automated Edman degradation was performed using an Applied Biosystems pulsed liquid sequencer model 473A (Weiterstadt, Germany) with on-line analysis of the phenylthiohydantoin (PTH) derivatives. The protein bands of interest were excised from the PVDF-blotted membrane using a razor blade. The membrane pieces were activated by wetting in 100% methanol, followed by 20% methanol. The membrane pieces were washed with distilled water, cut into smaller pieces and inserted into the slot of the sequencer cartridge. The sequence of the unknown protein was read by comparing the HPLC chromatograms of standard PTH-derivatised amino acids with those produced by sequential Edman degradation of the blotted protein fragment. Depending on the results obtained, 20-30 Edman degradation cycles were used. Edman degradation was also carried out on an Applied Biosystems Procise Sequencer model 494 (Applied Biosystems/Perkin Elmer, USA), with on-line analysis of the PTH derivatives. The same method was used as above, except that it was not necessary to activate or wash the membrane before cutting it into smaller pieces and inserting them in the sequencer cartridge.

Search for protein homologies

Comparisons of the sequences obtained with protein and DNA-derived portein sequence databases was carried out using an on-line connection to the European Molecular Biology Laboratory (EMBL) and MEDLINE links with the BLAST database via the Internet.

RESULTS AND DISCUSSIONS

Samples from 14 days post-slaughter were found to contain 110 kDa bands of the greatest intensity and were subsequently used for isolating and sequencing of the fragment. In comparison to the 30 kDa fragment the levels of the 110 kDa fragment that appear on SDS-PAGE over the ageing period are visually smaller. Concentrating the proteins in the myofibrillar extracts before SDS-PAGE increased the intensity of the 110 kDa band electroblotted onto the PVDF membrane for sequence analysis. Therefore, we were able to read an amino acid sequence of 27 residues and also confirm the residue assignment in positions which were previously unclear (Table 1).

Table 1. Sequenced primary structures of peptides from the 110 kDa protein fragment and homology with other proteins.

	Published result	Applied Biosystems Sequencer model 473A	Applied Biosystems Sequencer model 494
Bovine Peptide	110 kDa	110 kDa	110 kDa
Sequence	EQPEVDVWEL-X(T)X(S)NAL	EQPEVDVWEL-LKNAKPG(S/T)-G(Y/K)	EQPEVDVWEL-LKNAKPSEYE-KIAFQYG
Homologous peptide structure	C-protein (103-121, human)	C-protein (203-218, human, skeletal muscle slow-isoform)	C-Protein (204-219, human, myosin binding protein, slow-type muscle)
% homology	80%	77% for first 17 a.a.	81% for first 17 a.a.

All of the sequences read were found to share a common homology with human myofibrillar C-protein (m.wt. 140 kDa). This is a thick filament-associated protein, located in the crossbridge region of vertebrate striated muscle A bands. C-protein forms cylindrical structures around the packed tails of myosin molecules acting as a clamp to hold and stabilize the backbone of the thick filament (Bailey, 1982). It binds MHC, F-actin and native thin filaments *in vitro* and modifies the activity of actin-activated myosin ATPase (Furst et al, 1992). It may modulate muscle contraction or may play a more structural role. Studies investigating the effect of Ca^{2+} ions and proteinase inhibitors on myofibrils have shown that C-protein is solubilized by Ca^{2+} ions due to a salting-in effect rather than to proteolytic action by calpains (Taylor et al, 1991). Therefore, the solubilization of C-protein caused by the influx of Ca^{2+} ions into the muscle cells post-slaughter may be partly responsible for the weakening of the myofibrils and possibly increase their susceptability to proteolytic degradation.

Larger amounts of the known sequence for the 110 kDa peptide can now be produced synthetically for use as immunogen for antibody production. These antibodies may then be used to develop an immunoassay for detecting the 110 kDa protein fragment in a larger number of meat samples than by the original SDS-PAGE method. This will allow us to evaluate its' usefulness as a predictor of meat tenderness.

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