SEQUENCE ANALYSIS OF PROTEINS EXTRACTED FROM BOVINE MYOFIBRILLAR EXTRACTS DURING THE AGEING PERIOD

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INTRODUCTION

Although it is well accepted that beef undergoes a tenderisation process on ageing, the precise proteins involved in this mechanism remain unknown. Much evidence points to the role of the calpain system in the proteolysis of myofibrillar proteins during ageing. These proteins have not been identified. Recently we reported the partial elucidation of the sequence of the 30 kDa protein fragment as well as its protein of origin (Tsitsilonis et al., 1996). By internal microsequencing analysis the 30 kDa protein was found to be a fragmentation product of muscle troponin T. In this work we present data on the origin of 4 more fragmentation products of enhanced appearance with ageing, namely 16 kDa, 32 kDa, 90 kDa and 110 kDa, as well as the N-terminal sequence of the 30 kDa protein.

OBJECTIVES

The aim of this work was to identify the origin of some protein fragments which appear on SDS-PAGE profiles of myofibrillar extracts during the ageing period.

EXPERIMENAL METHODS

Preparation of samples:
The *M. longissimus dorsi* was excised from Hereford cross heifers at 24 h post-mortem and aged at 3±10°C for 14 and 21 days. Myofibrillar extraction and SDS-PAGE were performed according to Troy et al., (1986) at 2, 7, 14 and 21 days. Semi-dry protein transfer on Problott membranes (Applied Biosystems Inc.) was performed according to O'Halloran (1996). The amido-black stained proteins of interest were excised, dried and stored at room temperature until processed.
Sequence analysis:
Membrane pieces were activated by wetting them with 100% methanol, then 20% methanol in distilled water and finally with distilled water alone. The bands were cut in smaller pieces, inserted in the cartridge slot and sequenced on a 473A automated pulsed liquid protein sequencer (Applied Biosystems Inc.) equipped with an on-line 610A PTH analyser.

Search for protein homologies
Comparisons of the sequences obtained with protein and DNA-derived protein sequence databases was carried out with a CD-ROM (ATLAS Retrieval System, PIR-International Protein Sequence Database, Version 1996) and on-line to Swiss-Prot database through a modem and a PC compatible computer.

RESULTS AND DISCUSSION

These results demonstrate that bands appearing on SDS-PAGE gels of myofibrillar protein profiles during the ageing of meat originate from a variety of sources including both sarcoplasmic proteins and myofibrillar proteins (see Table 1). Troponin-T seems to be the origin of not only the 30 kDa band (75% similarity to the segment 32-51 of rabbit troponin-T) but also to a 32 kDa fragment. It seems that both fragments are N-terminal (90% similarity to the segment 24-33 of human troponin-T) degradation products of the same protein cleared at different sites i.e. at the N-terminus of residues 31 and 23 respectively. Furthermore since both fragments begin with a glutamic acid (Glu) residue and according to the primary structure of known mammalian troponin-T molecules, the preceding residue is also a Glu, it can be assumed that the protease responsible for troponin-T degradation in muscle could be either Glu-Glu or Glu-N specific. It also should be suggested that the full sequence of bovine troponin-T is not yet available.

The 110 kDa fragment revealed 80% similarity with amino acids 203-212 of human C-protein, a structurally important protein in the myofibril which serves to act as a clamp to hold and stabilise bundles of myosin molecules together in the thick filament (Bailey, 1982). The degradation of C-protein could partly result in the known ultrastructural destruction occurring in aged meat such as the disappearance of the transversal alignment of the Z-disc (Taylor et al., 1995).

The 16 kDa fragment is a N-terminal fragmentation product of glyceraldehyde-3-phosphate dehydrogenase (G-3-P-D), its sequence found identical to residues 1-15. The reported molecular weight of G-3-P-D in mammals is abut 35 kDa, therefore this fragment corresponds to the first half of this sarcoplasmic protein.

The 90 kDa fragment presents 80% similarity with the primary sequence of glycogen phosphorylase 21-30, a tetramenic enzyme of a report4ed 97 kDa molecular weight per subunit. Therefore the detected fragment could represent a single subunit deprived of the first N-terminal 20 residues.

The results presented here show that a number of bands relating to the ageing process can be identified with a high degree of confidence using sequence analysis. Other fragments are currently being assessed.

CONCLUSION

Fragments produced during the ageing process can be identified using sequence analysis. Troponin-T degradation causes the appearance of at least 2 fragments. Sarcoplasmic proteins undergoing degradation deposit fragments which are extracted with myofibrils.

Table 1: Comparison of sequenced primary structure segments of bovine proteins with known proteins

Bovine protein	Sequence found	Protein identified (origin)	% Homology
16 kDa	VKVGVNGFGRIGRLV	glyceraldehyde 3-phosphate dehydrogenase (1-15) (bovine)	100
30 kDa	EVHEPEEKPRP(R)RLT(L)AA(P)L(A)I(L)IE	troponin T (32-51) (rabbit)	75
32 kDa	EAPEE(V)PEP(E)AA(R)	troponin T (24-33) (human)	90
90 kDa	E(G)VENVTELX(M)X(L)	glycogen phosphorylase (21-30) (rabbit/human)	80
110 kDa	EQPEVDVWELX(T)X(S)NAL	C-protein (203-212) (human)	80

REFERENCES

Bailey, A.J. (1982). Muscle proteins and muscle structure. In: Food Proteins (P.F. fox and .J. Condon, eds.,). Applied Science Publishers, London and New York, 245.

O'Halloran, G. (1996). Influence of pH on the endogenous proteolytic enzyme systems involved in the tenderisation of beef. Ph.D Thesis, National University of Ireland.

Taylor, R.G., Gesink, G.H., Thompson, V.F., Koohmaraie, M. and Goll, D.E. (1995). Is Z-disc degradation responsible for post-mortem tenderisation? J. Anim. Sci., 73, 1351.

Troy, D.J., Tarrant, P.V., Harrington, M.G. (1986). Electrophoretic analysis of changes in beef myofibrillar proteins during the early post-mortem period. Biochem. Soc. Trans., 14, 436-438.

Tsitsilonis, O.E., Vazeou, S., Yialouris, P.P., Vandekerckhove, J., Stoeva, S., Voelter, W., Troy, D.J. (1996). Identification by sequence analysis of some critical (30 kDa) proteins in aged beef. Meat Focus Int., March, 77-79.