DEGRADATION AND RELEASE OF TITIN IN PORK MUSCLES

Pospiech E.[1,2], Greaser M.[3], Mikołajczak B.[1], Szalata M.[1], Łyczyński A.[4]

[1]Institute of Meat Technology, Agricultural University, Poznań, Poland, [2]Meat and Fat Research Institute, Poznań, Poland
[3]Muscle Biology Laboratory, University of Wisconsin-Madison, Madison, USA,
[4]Department of Animal Origin Raw Material, Agricultural University, Poznań, Poland

Background

Postmortem changes in muscles after slaughter are associated mainly with degradation of their structural proteins. The result of these processes is improved tenderness and other functional properties of meat. Endogenous enzymes are the main cause of the degradation. Calpains are believed to play a key role; however, the release of calcium ions from the sarcoplasmic reticulum and the changes in actin-myosin interactions may also influence the speed and extent of the degradation processes (Goll et al. 1997, Ouali 1992, Takahashi 1995). Not all muscle proteins are degraded by calpains (Bandman and Zdanis 1988, Hwan and Bandman 1989). It is assumed calpains weaken the cytoskeletal structure and this weakening is associated with degradation of proteins (Huff-Lonergan et al. 1996, Ho et al. 1996, Taylor et al. 1995). In some cases however degradation of cytoskeletal structure is followed by release of proteins; disassembly of the Z-disk structure with calpains releases α-actinin. Investigations of Boles et al. (1992) revealed that the degradation of titin in pork is slower in muscle from stress-positive than in muscle from stress-negative animals. Similar phenomena were observed in turkey breast muscles (Pospiech et al. 1997) and were also associated with lower protein solubility (Pietrzak et al. 1997). Other studies (Pospiech et al. 1996) showed that small amounts of structural proteins were released during very fast post mortem glycolysis. Heretofore information related to the liberation of cytoskeletal proteins from the muscle tissue structure is limited other than as a result of salt treatment (Paterson et al. 1988, Wang and Greaser 1985, Greaser et al. 1997). It was suggested that determination of quantities of a selected protein in centrifugal drip could be used as a simple test for changes in the meat structure after salt treatment and a measure of functional properties of meat (Grześ et at. 1996).

Objective

The aim of this study was to observe cytoskeletal protein degradation in pork muscle and identify the high molecular weight protein components released in the centrifugal drip during post-mortem storage.

Materials and Methods

The lumbar regions of loins from 7 pork carcasses were removed at 24 hours after slaughter. The selection of muscles was performed on the basis of pH_1 (45 minutes) and pH_2 (24 hours after the slaughter) measurements. Only normal quality muscles (pH_1 > 5.8 and pH_2 < 6.0) were used in this study. Each muscle was divided into four parts, placed in vacuum bags, and stored in a cold room at 2 – 4°C for two weeks. Observation of titin degradation in the muscle and its release from the cytoskeletal following centrifugation was carried out at 48, 120, 168 and 336 h after the slaughter.

Myofibrils were prepared according to the procedure described by Fritz and Greaser (1991) with slight modifications. A 2 g of muscle sample was homogenised in 20mL of rigor buffer (50mM KCl, 50mM Tris (pH 7,5), 5mM EGTA, 2mM NaN_3, 0,1mM leupeptin, 1mM benzamidine, 0,1mM PMSF and 0,5% Triton X-100) and after it centrifuged at 1000 g for 10 minutes. The myofibrils pellet was resuspended in fresh rigor buffer (without Triton X-100), homogenised and centrifuged at the same conditions as before. After this extraction washed myofibrils were resuspended in equal volumes of glycerol and rigor buffer (from the second washing) and stored at –20°C until future use. Centrifugal drip was collected by centrifugation of meat samples (about 8g) at 15000g for 20 minutes at 2°C. The supernatant, referred later to centrifugal drip, was retained for electrophoretic analysis. Proteins of washed myofibrils and centrifugal drip were analysed using the polyacrylamide gel electrophoresis (15% acrylamide, pH 8.8 of resolving gel and 8M urea) and agarose gels (1.5% of SeaKem Gold agarose, pH 8.5) according to the method adapted from Wu and Kusukawa (1998). The protein quantitation on gels was determined using a GS 300 Transmittance/Reflectance Scanning Densitometer (Hoefer). Western blotting was conducted according to the method of Fritz and Greaser (1991) also with slight modifications, which concerned mainly the transfer of proteins. Proteins were transferred on 0.45 μm Immobilion – P (Millipore) membrane using SemiPhore™ Semi-Dry transfer unit from Hoefer®. A transfer buffer of 10mM CAPS (pH 11.0) containing 10mM 2-mercaptoethanol and 0.1% (w/v) SDS was used instead of Towbin buffer. Primary antibodies used included anti-titin monoclonal 9D10 cell supenatant diluted 1:600 and anti-titin monoclonal 856 purified ascites diluted in the ratio of 1: 20000 in 0.2%BSA in TBST.

Results and discussion

Measurements of pH at 45 minutes and 24 hours post-mortem gave mean values of 6.48 and 5.41 respectively. During subsequent storage the pH value changes were small and statistically insignificant. Electrophoresis of proteins from centrifugal drip revealed a significant amount near the top of the gels with molecular weights above 200kDa. Densitometer measurements showed that these proteins constituted 0.9 to 1.5% of the total protein of the drip at 48h after slaughter. At 120 h the amount of these high molecular weight proteins (HMP) increased to 0.6 – 2.5%. The values further increased to 1.5 - 7.2 and 1.8 – 13.1% at 168 and 336 hours respectively. Electrophoretic separations also revealed an increased number of bands with storage time, especially in the high molecular weight range. In some cases however considerably higher amount of these proteins was found in samples taken 168 h after the slaughter instead of measurements performed at the last stage of the study. Observation of myofibrillar protein separation on 1.5% agarose gels showed that up to 168 h after the slaughter both the T1 and T2 forms of titin were observed. A region of protein staining that migrated below the position of the titin T2 was found in the centrifugal drip at 120 h after the slaughter. Later, after 168

and 336 hours, not only these small degradation products, but also the T2 form of titin was found. Figure 1 presents the blot developed with the use of anti – titin monoclonal antibody 856, which does not recognise the titin degradation product of 1200kDa . The reaction with 9D10 (not shown in this paper) confirmed that smaller fragments of titin were present in centrifugal juice. Electrophoretic separations of centrifugal juice showed also, that the longer time of storage, the larger titin degradation products were observed on gels. Longer storage favours increased high molecular weight protein into the drip of meat that contained higher amounts of the titin. These observations revealed that the weakening of cytoskeletal structure in pork longissimus dorsi is caused not only by the degradation of titin but also its release from the myofibrillar complex. Titin degradation and its release from muscle's cytoskeletal structure may be the cause of increased tenderness of meat and its improved functional properties with meat ageing.

Conclusions

1. Prolonged storage of meat favours increased release of high molecular proteins into the drip
2. Post mortem changes of titin are associated with its degradation and release from the cytoskeletal structure of meat
3. Larger degradation products of titin, including its T2 form, appear in centrifugal drip after prolonged storage

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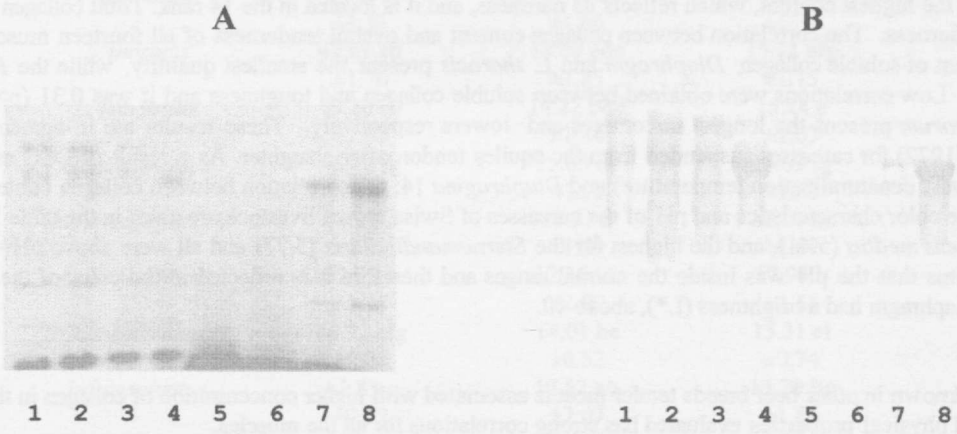
Figure 1. Separation of myofibrillar proteins and corresponding centrifugal drip samples from m. longissimus dorsi



Set A. 1,5% SeaKem Gold agarose gel; Lane 1 - myofibrils from meat taken 48h after the slaughter, 2 – myofibrils 120h, 3 – myofibrils 168 h, 4 - myofibrils 336 h, 5 – centrifugal drip collected 48h after the slaughter, 6 – drip 120h, 7 – drip 168 h, 8 – drip 336h

Set B. Imunoblot using monoclonal anti-titin 856. The lanes and samples correspond to the electrophoretic separation in the set A.