Session 4.I

Biochemistry and functional properties of meats

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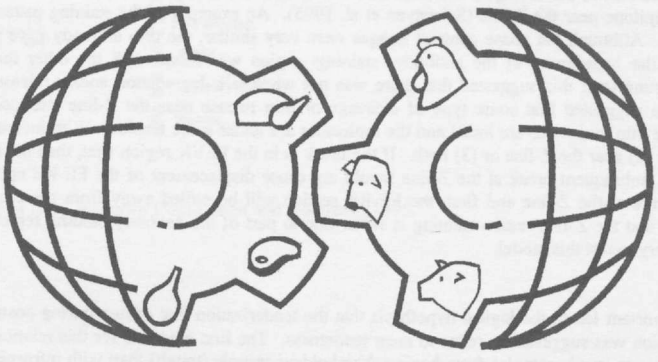

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TITIN AND TENDERNESS

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Background

The protein titin, also called connectin, is a giant protein in muscle tissue (Maruyama 1976; Wang, et al. 1979). It was early recognized that this protein was very susceptible to proteolytic degradation (Wang 1982; Maruyama, et al. 1984; Trinick, et al. 1984) with the parent molecule (T1 or α-connectin) being converted to a smaller protein species (T2 or β-connectin). This proteolytic conversion has been of great interest in regard to the function of titin in living muscle, but the conversion and the effects of titin breakdown postmortem have also received much attention from meat scientists. The current paper will review previous work on postmortem breakdown of titin as it relates to meat tenderness. Additional results from recent work in our laboratory will also be presented regarding titin changes in meat.

Our understanding of titin structure was greatly aided when Labeit and Kolmerer (1995) published the full cDNA sequence of titin. A diagram of the domain structure of the titin is shown in Figure 1. Most of the protein consists of approximately 100 amino acid motifs of two types: ones similar to immunoglobulin domains (Ig) and others similar to fibronectin type 3 domains (FN3). Over 250 of these domains are present in skeletal muscle titin. Both types of domains are made up of seven stranded beta sheets. The titin molecules are aligned in two sets parallel to the long axis of the myofibril with the amino terminus in the Z line and the carboxyl terminus at the M-line. Thus titin runs the full length of the sarcomere. Starting from the Z line, a group of 10 Ig domains is assembled with several unique intervening sequences not found in other proteins. Proceeding toward the middle of the sarcomere, a group of Ig domains is merged in a continuous pattern refered to as the 1st tandem Ig region. The length of this 1st tanden Ig region varies between 15 for the shortest form of cardiac titin to 68 in the soleus skeletal muscle. Next comes a unique sequence area that corresponds to the position of the N2 lines sometimes seen in electron microscopic images of muscle. Adjacent to the N2 region is a zone referred to as the PEVK, so named because over 70% of the amino acids consist of proline (P), glutamic acid (E), valine (V), and lysine (K). The PEVK is believed to extend when muscle is stretched (Trombitas, et al. 1998b). Next comes another set of consecutive Ig domains in a section referred to as the 2nd tandem Ig. Then at the junction between the I-band and A-band a set of FN3 domains appear. At the edge of the A band a series of six repeats consisting of one Ig, two FN3, one Ig, three FN3 occurs in the D zone (D=distal). Then follows a large zone of the A-band with 11copies of an 11-domain super-repeat referred to as the C zone (this region corresponds to the area where C protein occurs in the sarcomere). Adjacent are a few more Ig and FN3 domains and a serine/threonine protein kinase domain. Finally additional Ig domains and several unique sequence segments complete the carboxyl terminus at the M-line. A single gene codes for multiple titin isoforms; variations are primarily in the lengths of the PEVK (163 residues in the N2B cardiac isoform and 2174 in the soleus skeletal muscle) and the first tandem Ig region (15 to 68 Ig domains) (Labeit and Kolmerer 1995).

Postmortem Titin Degradation

The breakdown of titin postmortem was first reported by Takahashi and Saito (1979) who reported the amount of connectin they could isolate from chicken breast muscle declined with time after death. Later it was demonstrated that the electrophoretic T1 titin band (the one with slowest mobility) was most prominent in bovine muscle and myofibrils isolated soon after death, but during the subsequent seven days postmortem the intensity of the T1 band declined while the T2 band (faster mobility corresponding to a smaller molecular weight) became the dominant titin species (Lusby et al. 1983). Similar results were obtained using improved electrophoretic methods (Fritz and Greaser 1991; Huff-Lonergan et al. 1995). The rate of titin T1 to T2 conversion was affected by postmortem holding temperature with rates at 37°C > 25°C > 2°C (Lusby et al. 1983). The titin splitting was shown to occur in the I-band and also generate a fragment of approximately 1200 kDa in size (Matsumura et al. 1991; Tanabe et al. 1994).

There also appears to be a structural re-arrangement of titin during the postmortem time period. Staining of myofibrils with the titin monoclonal antibody 9D10 (Wang and Greaser 1985) normally shows two bands per sarcomere in myofibrils isolated from bovine muscle within the first few hours postmortem. However, the pattern is changed to give four bands per sarcomere in many bovine psoas myofibrils by two days postmortem (Ringkob et al. 1988). A more detailed study showed that the proportion of psoas myofibrils giving the four-band pattern increased to approximately 65% by 2 days postmortem and remained constant until two weeks after death (Fritz and Greaser 1991). The band pattern re-arrangement paralleled the conversion of T1 to T2 that was complete within the same time period. The conversion of the titin band pattern was not an artifact of the myofibril isolation; similar four-band staining patterns were also found in sections of the intact muscle tissue (Fritz and Greaser 1991). The positions of the two bands in each half sarcomere were different than the single band; it appears that the two parts of the titin retract in opposite directions (Fritz and Greaser 1991).

A clearer understanding about the location of the cleavage point in titin has been determined recently in our laboratory. Previous results had suggested that titin breaks at the N2 line position (Kimura et al. 1992; Kawamura et al. 1995). A pair of titin antibodies (9D10 and 514) was used to stain bovine psoas myofibrils isolated at 48 hours postmortem (Figure 2). The 9D10 has been shown to bind to multiple sites in the PEVK region (Trombitas et al. 1998b). The 514 antibody was prepared against a synthetic peptide corresponding to the A-band end of the PEVK region (Trombitas et al. 1998a). The figure shows that the 514 labels a single band in each half sarcomere while the 9D10 binds two bands. These results suggest that the true titin cleavage location is in the PEVK rather than in the N2 region. This observation is consistent with the fact that the PEVK would be more susceptible to proteolytic cleavage because of its flexibility. These results also explain the reason for the appearance of the extra 9D10 stained bands that result from postmortem storage.

Recent evidence suggests that there may be titin re-arrangement near the Z line (Boyer-Berri and Greaser 1998). Myofibrils were prepared from four different bovine muscles (cutaneous trunci, rectus abdominis, psaos major, and masseter) at six different time periods (0, 1, 2, 4, 8, and 16 days) and subsequently stained with the FE-RE antibody (raised against a titin epitope near the Z-line (Sebestyen et al. 1995). An example of the staining patterns of psoas myofibrils obtained at 0, 2, and 16 days postmortem is shown in Figure 4. Although the phase contrast images were very similar, the titin antibody gave progressively wider antibody staining zones with increasing time postmortem. A similar broadening of the antibody- staining- region was found with the other three muscles as well. The total amount of fluorescence did not decline with time postmortem; this suggested that there was not wholesale degradation and/or release of the titin segment near the Z-line. The widening of the fluorescently stained zone suggested that some type of cleavage or titin release near the Z-line was occurring. An interpretation of these pattern changes is shown in Figure 5. At 0 day the titin molecules are intact and the molecules are under some tension. With increasing time postmortem, cleavage could occur in the titin at either (1) the PEVK region or (2) near the Z-line or (3) both. If the break is in the PEVK region first, then the broken titin on the A-band end may collapse back to the ends of the thick filaments. A subsequent break at the Z-line would not cause displacement of the FE-RE epitope since this segment of titin is no longer under tension. However, if the break occurs at the Z-line end first, the FE-RE section will be pulled away from the center of the Z-line. By 16 days postmortem essential all the titin molecules are broken and the Z-line region staining is wider due to part of the antibody binding regions being pulled away from the Z-line edge. Electron microscopic studies will be necessary to test this model.

Titin Degradation and Tenderness

Observations of titin breakdown postmortem led to the logical hypothesis that the tenderization that occurs during postmortem aging is due to titin breakdown. In addition the extent of the T1 to T2 conversion was suggested to relate to meat tenderness. The first evidence for this relationship was presented by Paterson and Parrish (1987). They observed that titin breakdown was less in samples from bovine rhomboideus muscle (tough) than with infraspinatus muscle (tender). It was further shown that electrophoretic samples from tough bovine longissimus muscle had a higher intensity of titin staining than those from more tender samples (Anderson and Parrish 1989). Subsequent studies demonstrated that the changes in titin degradation patterns comparing muscle from steers and bulls versus cows paralleled the tenderness patterns of these groups (Huff-Lonergan et al. 1995). Furthermore, within group comparisons were shown indicating that tougher steer samples had higher titin T1 proportions than those from tender muscle. Additional support for the titin-tenderness relationship was provided by studies indicating that animals with Bos indicus breeding had greater toughness and slower postmortem titin degradation than samples obtained for the British breeds (Ho et al. 1997). Double-muscle animals also had slower titin degradation rates and greater meat toughness (Uytterhaegen et al. 1994). The Callipyge sheep has slower titin degradation rates and tougher meat (Geesink

and Koohmaraie 1999). All these studies support the theory that postmortem conversion of titin T1 to T2 or smaller fragments is highly related to the tenderness properties of meat.

If the degradation state of titin is a major factor affecting tenderness, then animals with similar breeding and age should show a relationship between the tenderness of the meat and the extent of titin degradation. A major study conducted in our laboratory several years ago, however, did not yield data that was consistent with the titin degradation – tenderness hypothesis (Fritz et al. 1993). A set of 24 Chianina cross-bred steers that had been finished in the same feedlot were slaughtered and samples from the Longissimus removed at 2 days and 16 days postmortem for both Warner-Bratzler shear measurements and polyacrylamide gel electrophoresis. Both whole muscle and isolated myofibril samples were used for electrophoresis. Great care was taken to fix the proteins in the gels before staining and to measure the quantities of stained gels in the linear range of load versus staining intensity. The ratio of the titin bands to the myosin heavy chain was determined for each gel lane to correct for potential differences in sample loading. The 16-day samples showed more T2 than the 2-day samples and these results paralleled the overall improvement of tenderness that occurs during aging. However, when samples were compared within a postmortem time period the relationship between proportion of titin and tenderness was not significant. Since samples for shear determination are cooked, it was possible that changes in titin might occur during the cooking procedure. Thus samples for electrophoresis were also prepared from the cooked steaks. Again the titin proportion (relative to the myosin heavy chain) declined during cooking, but the total titin showed no significant relationship to the tenderness of the meat when samples were compared within the same treatment group. In addition the ratio of titin T1 to T2 did not relate to meat tenderness. Differences in sample preparation, electrophoresis acrylamide and buffer conditions, sampling times postmortem, and genetic background of the animals examined might all be related to conclusions counter to previous work.

We have recently developed modified procedures for electrophoresis of high molecular weight proteins using agarose instead of acrylamide. A photograph of one of these gels is shown in Figure 3. The samples shown are from pig longissimus taken within a few minutes after death and after 5 days of postmortem storage. The titin T1 and T2 bands are much better separated than when acrylamide gels systems are used. Use of this system should help to resolve questions regarding the relationship of titin degradation to meat tenderness.

Titin Release from Myofibrils Postmortem

Recent work has shown that titin may be partially released from its myofibril attachments in postmortem tissue. Porcine muscle, at various times up 14 days postmortem, was subjected to centrifugation at 15,000 X G. Surprisingly the resulting centrifugal drip showed a significant amount of high molecular weight bands on SDS polyacrylamide gels. Western blotting with titin monoclonal antibodies demonstrated that titin T2 and smaller titin fragments were present in this drip at 7 and 14 days postmortem. In contrast there was no evidence for myosin heavy chain release with time postmortem. Titin T2 is normally only soluble in higher salt concentrations than found in muscle. Of the proteolytic titin fragments, the 1200 kDa would be the most soluble at physiological ionic strength (Matsumura et al. 1991). Thus the appearance of titin T2 released in the drip was unexpected. The significance of this finding in relation to tenderness and meat quality is currently under investigation.

Conclusions

The role of titin in meat tenderness has been examined in numerous studies, and there is strong evidence that titin is degraded to smaller pieces during the same postmortem time frame as tenderization occurs. However, experiments involving large sets of animals with the same genetic background, same animal age, and same postmortem time period have been more limited. Thus whether titin degradation state is a primary factor in determining meat tenderness or only a secondary indicator will require additional research. Titin is broken in both the PEVK region and near the Z-line during postmortem storage. There also appears to be a loosening of the attachment of titin to the thick filaments during postmortem aging.

Acknowledgments

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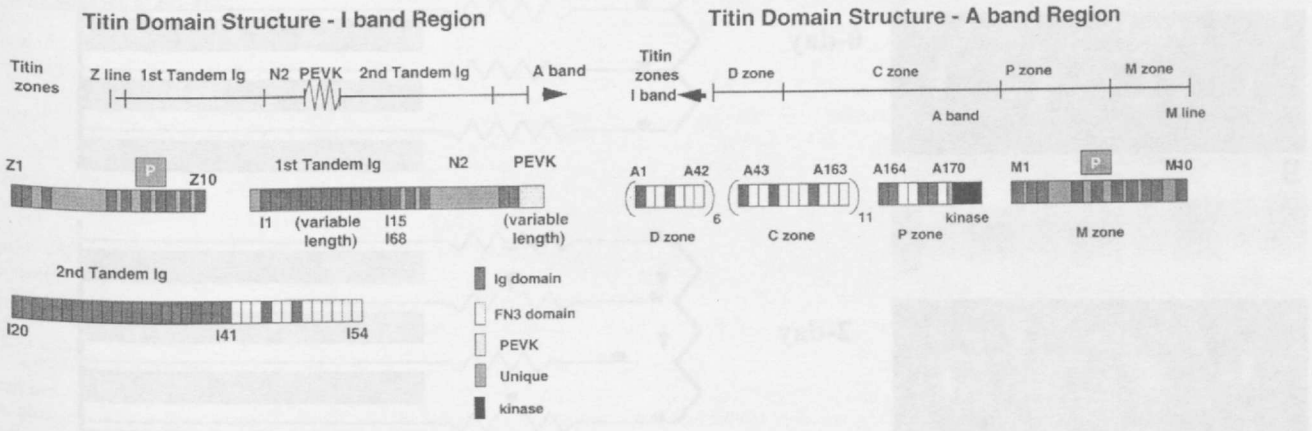

Figure 1. Diagram of the domain structure of titin. Redrawn from Labeit and Kolmerer, 1995.

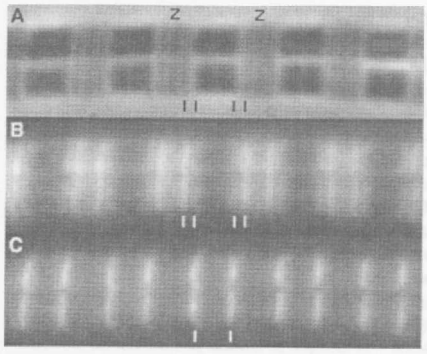

Figure 2. Titin antibody localization in 48-hour postmortem bovine psoas muscle. Top – phase contrast. Middle – 9D10 monclonal staining. Bottom – antibody 514 staining. The extra 9D10 bands that appear postmortem are derived because titin is cleaved in the PEVK region, there are multiple 9D10 antibody binding sites, and the two segments of the PEVK retract away from each other. Vertical bars are in equivalent positions in the upper and middle panels; the bars in the lower image are in the same position as the two inner bars from above. The sarcomere length (Z to Z distance) is approximately 3.5 microns.

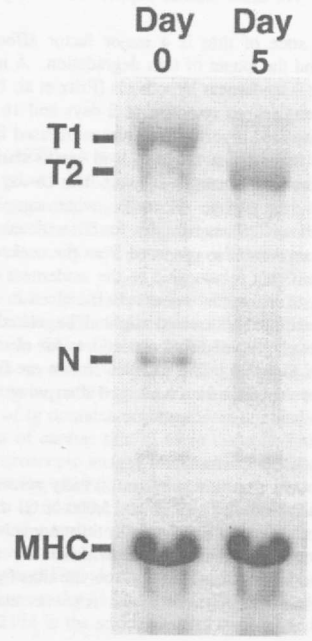

Figure 3. Agarose gel electrophoresis of the high molecular weight proteins from porcine muscle at death and after 5 days postmortem storage. The titin T1 and T2 are much better separated on this gel system compared with polyacrylamide gels.

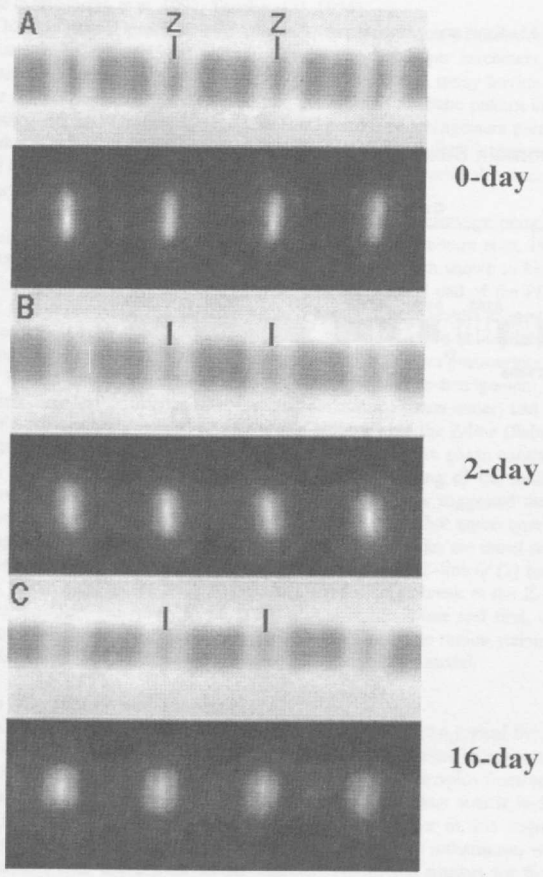

Figure 4. Postmortem changes in titin near the Z-line. Myofibrils were prepared from bovine psoas muscle at 0, 2, and 16 days postmortem and stained with the FE-RE antibody. Note that the width of the fluorescent band band near the Z-line increases with storage time. Adapted from Boyer-Berri and Greaser, 1998.

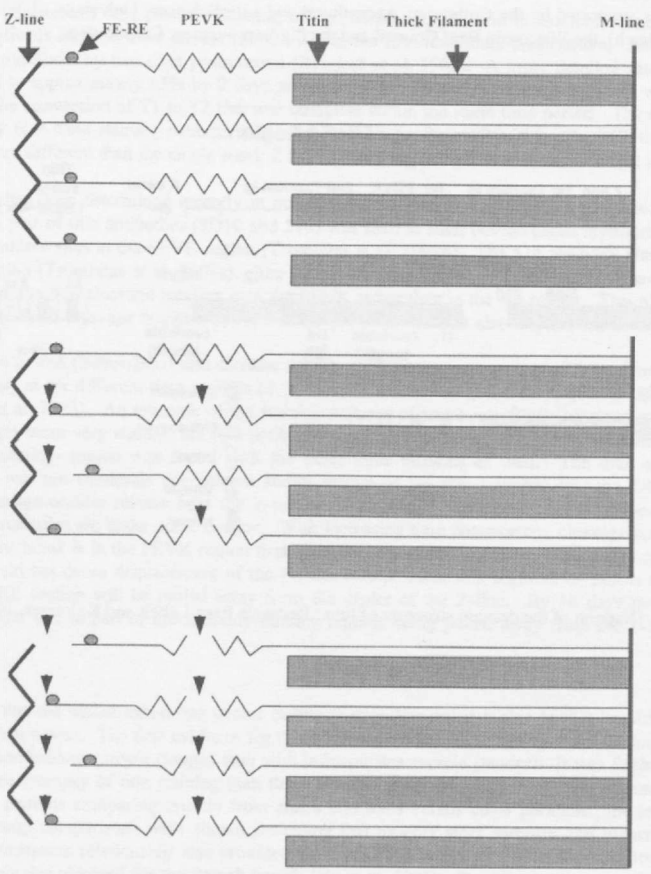

Figure 5. Interpretative diagram of changes that occur in titin during postmortem storage. Arrows indicate potential titin break points near the Z-line and in the titin PEVK region. The thin filaments are omitted in the diagram for clarity. Adapted from Boyer-Berri and Greaser, 1998.

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