TRANSMUTATION IN TALIN2 AND THE QUALITY OF CHICKEN MEAT

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Abstract—**The cytoskeletal protein talin serves as an essential link between integrins and the actin cytoskeleton in costameres. Some researchers have reported that talin may contribute to the water-holding capacity of aged meat. There are two isoforms of talin, Talin1 (T1) and Talin2 (T2) and whether their biological roles differ from one another is not fully understood. Furthermore, the specific roles of T1 and T2 with respect to their direct involvement in meat quality are unclear. In this report we have analyzed the expression of T1 and T2 in the pectoralis major, biceps femoris, gizzard and the heart in chicken, and the postmortem change of T2 in pectoralis major. Using real time PCR and western blotting, we found that T2 is more highly expressed than T1 in the pectoralis major, biceps femoris and the heart and that their expressions in the gizzard are approximately equal. T2 in chicken biceps femoris was degraded during postmortem storage. Consequently, we propose that the degradation of T2, but not T1, in chicken skeletal muscle may influence the quality of chicken meat.**

Index Terms—**chicken meat, cytoskeletal proteins, meat quality, Talin1, Talin2**

I. INTRODUCTION

The intact talin protein is 270 kDa (2541 amino acids) and can be proteolyzed between residues 433 and 434 by the calcium-dependent protease calpain II to generate an N-terminal head domain and a C-terminal rod domain (Rees et al., 1990). Talin is found in several muscle-specific, integrin-linked adhesion complexes, including costameres, intercalated disks and myotendinous junctions. Costameres link actin-containing Z-disks to the sarcolemma through integrins in skeletal and cardiac striated muscle. The talin head domain contains integrin binding sites (Garcia-Alvarez et al., 2003) and the rod domain contains actin binding sites (Muguruma, Nishimuta, Tomisaka, Ito and Matsumura, 1995).

It has been reported that postmortem degradation of muscle proteins is associated with meat tenderness (Koohmaraie, 1992) and water-holding capacity (Huff-Lonergan and Lonergan, 2005). The calcium-dependent proteases, μ-calpain (calpain I) and m-calpain (calpain II) play a major role in proteolysis of muscle proteins under postmortem conditions (Huff-Lonergan and Lonergan, 2005). It is known that μ-calpain degrades intermediate filament proteins and costameric proteins (including desmin, vinculin and talin) in postmortem muscle.

Vertebrates have two separate talin genes, TLN1 and TLN2, which are found in all available vertebrate genomes (Senetar and McCann, 2005). Several studies suggest that T1 and T2 have non-redundant roles in vertebrates. As described above, the degradation of the costameric protein talin may play a role in the water-holding capacity in meat. However, until recently, most studies in meat science have focused on the function of talin as a single protein. The objective of the experiments described here was to determine the expression and the postmortem changes of T1 and T2 in chicken muscle in view of their influence on meat quality.

II. MATERIALS AND METHODS

mRNA levels of T1 and T2 in chicken pectoralis major, biceps femoris, gizzard and heart were determined by real-time PCR using the Ct method and normalized to GAPDH. The primers used in this study are shown in table 1. mRNA levels of T1 in each muscle are shown relative to T2, the level of which was arbitrarily assigned the value of 100% in each muscle.

For western blotting, the antibody clone 8d4 and anti-Talin2 were used for labeling T1 and T2, respectively, in chicken muscles. Chicken biceps femoris were stored at 4 ºC for 1-5 days after slaughter. The meats were taken out on 1, 2, 3, 4 and 5 days and homogenized at each time point. These samples were subjected to western blotting using antibody anti-Talin2.

Table 1 Primers of Talin1, Talin2 and GAPDH.

Primer	5' to 3' sequence
Talin1 forward	TGATGGTCACCAACGTCACT
Talin1 reverse	GAGAAGACCGCCAGTTCTTG
Talin2 forward	AGCTGCAACCAGCAATCTTT
Talin2 reverse	GCGACTTGTTTTGCAGATGA
GAPDH forward	GACGTGCAGCAGGAACACTA
GAPDH reverse	CTTGGACTTTGCCAGAGAGG

III. RESULTS AND DISCUSSION

In quantitative analysis, mRNA levels of T1 and T2 in chicken muscles are expressed as percentages and shown in figure 1. The mRNA level of T2 was significantly higher than that of T1 in the pectoralis major, biceps femoris and the heart, while in the gizzard, the mRNA level of T1 was slightly higher than that of T2.

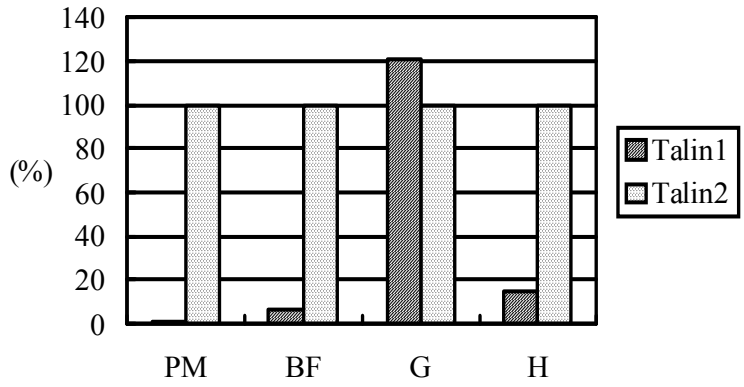

Figure 1: mRNA levels of Talin1 and 2. PM: pectoralis major, BF: biceps femoris, G: gizzard and H: heart. mRNA levels were determined by real-time PCR using the Ct method and normalized to GAPDH. mRNA levels of Talin1 in each muscle are shown relative to Talin2, which was arbitrarily assigned the value of 100% in each muscle.

Antibody 8d4 strongly labeled a double band around 270 kDa, showing the presence of intact talin in heart samples, which was hardly detectable in skeletal muscles. However, a single band was strongly labeled in gizzard by 8d4 (Fig. 2A). 8d4 also labeled a band 'a' that may be a degraded product in the gizzard. Anti-Talin2 antibody labeled a single band around 270 kDa in the pectoralis major and biceps femoris and was more obvious in the heart (Fig. 2B), while a double band was apparently labeled in gizzard by anti-Talin2. Anti-Talin2 strongly labeled the bands 'a' and 'b', which may be the degraded products of intact talin in the pectoralis major and biceps femoris.

In general, the bands labeled by 8d4 were labeled more weakly than the bands labeled by anti-Talin2 in the pectoralis major, biceps femoris and the heart. However, the strength of band labeling by 8d4 and anti-Talin2 were equal in the gizzard. This suggests that the antibody 8d4 labeled chicken T1 and that anti-Talin2 labeled chicken T2, because it was shown that the mRNA level of T2 was higher than T1 and that the bands labeled by anti-Talin2 were stronger than the bands labeled by 8d4 in chicken skeletal muscle. This may suggest that T2 is more highly expressed than T1 in chicken skeletal muscle. Accordingly, we considered it important to examine the postmortem changes of T2 in chicken skeletal muscle using antibody anti-Talin2.

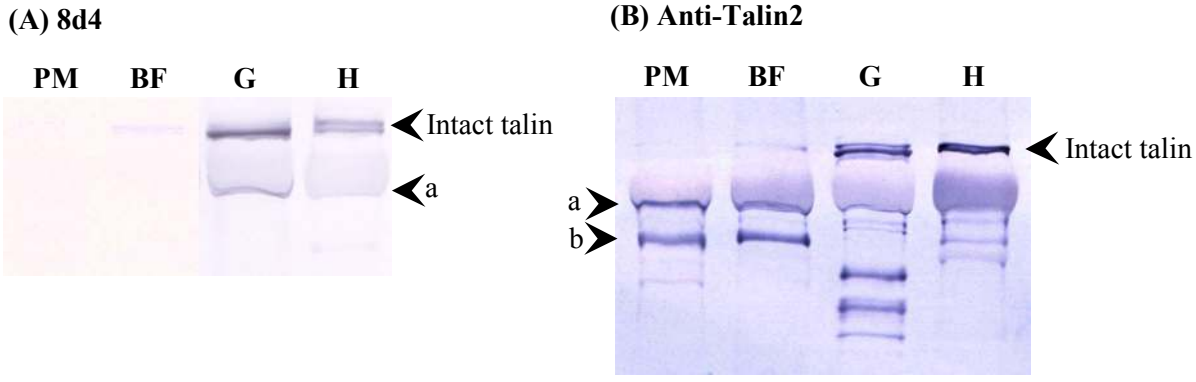

Figure 2: Western blotting of talin in the pectoralis major (PM), biceps femoris (BF), gizzard (G) and the heart (H) in chicken. Antibodies (A) 8d4 and (B) anti-Talin2 was used for labeling T1 and T2, respectively, in each muscle.

Figure 3 shows the postmortem change in proteins (tentatively identified as T2) labeled by anti-Talin2 in chicken biceps femoris during 5 days storage at 4 ºC. A 270 kDa band was labeled by anti-Talin2 after 1 day of postmortem storage. However, this was absent after 2-5 days. It seems that the degradation of T2 was faster than that of desmin and vinculin (data not shown). It is likely that the enzyme which degrades T2 is different from the enzyme which degrades desmin and vinculin. Talin is a substrate for calpain II, as described above, whereas Senetar et al. (2007) reported that T1 is a substrate for calpain II, but that T2 may not be. Meanwhile, μ-calpain (calpain I) is an essential enzyme for degrading muscle proteins during postmortem storage (Geesink et al., 2006). In the current study, it was shown that the band patterns of skeletal and cardiac muscle talin were different from that of smooth muscle. This may suggest that the enzyme system which degrades talin in skeletal and cardiac muscle during postmortem storage is different from that in smooth muscle and indicates that endogenous enzymes differ in their biological roles. In other words, it may suggest that the biological roles of T1 and T2 in skeletal and cardiac muscle differ from those in smooth muscle in chicken. There is a need to identify the enzyme that degrades T2 during postmortem storage. The early postmortem degradation of T2 that links the Z-disk and the sarcolemma may mean decreasing lateral shrinkage and the creation of space between myofibrils and the sarcolemma. It is likely that the degradation of T2 influences the quality attributes in chicken meat, including tenderness and water-holding capacity in the early postmortem stage.

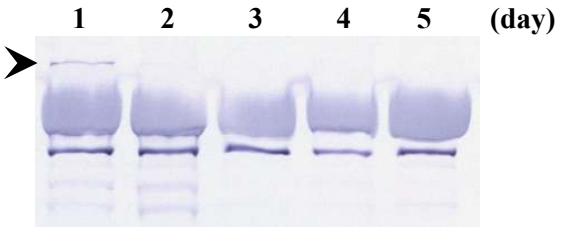

Figure 3: Western blotting of Talin2 in biceps femoris in chicken using antibody anti-Talin2. Chicken biceps femoris were stored at 4 ºC for 1-5 days after slaughter.

IV. CONCLUSION

The mRNA level of T2 was higher than T1 in the pectoralis major, biceps femoris and the heart. However, in the gizzard, the mRNA levels for T1 and T2 were approximately equal. The clarity of the bands labeled by 8d4 was lower than that in the bands labeled by anti-Talin2 in the pectoralis major, biceps femoris and the heart. These results suggest that T2 is expressed at higher levels than T1 in skeletal and cardiac muscle and that difference was remarkably observed in the western-blotting data of antibodies 8d4 and anti-Talin2. It was shown that T2 in the skeletal muscle is degraded early postmortem. The degradation of T2 may impact the quality characteristics of fresh chicken meat.

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