Does postmortem proteolysis depend on fibre type distribution ?

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Background

Previous investigations showed that proteolytic degradation occurred faster in white muscles than in red muscles (Whipple & Koohmaraie, 1992). Variation in eating quality between muscles have often been correlated to the metabolic properties, as determined by the fibre type distribution. However, correlation's between the fibre type distribution and the degree of postmortem proteolysis could result from two possible effects: (1) Due to inherent differences in metabolic potential, composition and content of proteolytic enzymes, fibres of some types may degrade more than others. (2) The balance of fibre types controls postmortem metabolic characteristics of the muscle as a whole, with all fibre types within it being equally affected. So far, no studies have investigated the differences in rate of proteolysis at the level of single fibre types. An experiment was conducted to compare the rate of postmortem proteolysis in five porcine muscle differing in fibre type distribution and to compare the rate of proteolysis in type II fibres isolated from these muscles.

Methods

Semitendinosus (ST), Semimembranosus (SM), Longissimus dorsi (LD), Soleus (S) and Vastus intermedius (VI) were excised immediately after slaughter, from three female (crossbreeds of Duroc, Landrace and Yorkshire) pigs. ST, SM and LD represent white muscles, VI a red muscle, and S is considered as a mixed muscle. At 1 hour postmortem (p.m.) ST, SM, LD, S and VI were removed from one side of the carcass. The muscles were cut into smaller samples, vacuum packed and immediately frozen in liquid nitrogen. At 24 h p.m. the five muscles from the opposite side of the carcass were removed and the muscles divided into three parts. One part was randomly chosen and treated as mentioned above. The two other parts of the muscle were stored for 2 and 7 days at 2 °C respectively. After storage these parts were cut into smaller samples which were immediately frozen in liquid nitrogen.
Muscle fibre types were identified by staining for myofibrillar ATPase activity (Brooke & Kaiser, 1970).

Degradation of two myofibrillar proteins; desmin and troponin-T, were determined on muscle homogenates and isolated type II fibres prepared from ST, SM, LD, VI and S muscles of animals at day 0, 1, 3 and 8 p.m. The samples were dissolved in buffer containing 4M urea, 1M thiourea, 0.025M Tris-base, 1% DTT and 1.5% SDS (pH 6.8) (Fritz et al., 1989). Myofibrillar proteins were separated by SDS-PAGE on 10% Bis-Tris gels (Nupage, Novex, CA, USA). The amount of protein loaded in each lane was 15 µg for muscle homogenates. In order to correct for protein loading on the single fibre blots, a skeletal anti-actin antibody (Sigma) were used on the assumption that actin concentration is essentially independent of ageing time. Protein bands were identified by Western immunoblotting using specific antibodies against desmin (DE-R-11, 1:5.000, Dako) and troponin-T (1:10.000, Sigma). Protein bands were visualised after incubation with BCIP/NBT. Densitometric scans of immunoblots were performed using the CREAM software program (Kem-En-Tek, Denmark). Data were analysed using the General Linear Models (GLM) procedure of SAS (SAS, 1988). The model included the fixed effects of muscle, ageing time and their appropriate interactions.

Results and Discussion

Porcine LD, SM, ST, VI and S muscles exhibit different fibre type distributions. LD, SM and ST contains predominantly type IIb fibres (> 81%), VI consist predominantly of type I fibres (80%) and S contains around 23% type I and 58% type IIb fibres (figure 1). ST tended to have a lower proportion of type I fibres (p= 0.09) and a higher proportion of type IIb fibres (P=0.09) than LD but the fibre type distribution of ST was not significantly different from the fibre type distribution of SM.

Examination of whole muscle homogenates revealed that the rate of postmortem proteolysis of desmin and troponin-T varied between the muscles (figure 2). In general, the rate of degradation of the two proteins occurred faster in LD and SM than in ST, S and VI. Degradation of troponin-T resulted in the appearance of two degradation products with molecular weights of 32 to 30 kDa (figure 2a), whereas degradation of desmin resulted in the appearance of three degradation products with molecular weights of approx. 50, 47 and 39 kDa (figure 2b). The rate of proteolytic degradation of desmin and troponin-T was expected to be similar for the three white muscles (ST, LD and SM) because the fibre type distribution of these muscles did not vary significantly (figure 1). However, the results showed that ST exhibited the same rate of degradation as VI and S, even though VI and S has markedly different fibre type distributions compared to ST. The inter-muscle differences in the rate of proteolytic degradation can therefore not be explained solely by the fibre type distribution but may also be influenced by other muscle-specific traits independent of the fibre type classification used in this study (e.g., proteolytic potential i.e., µ-calpain:calpastatin ratio).

Proteolytic degradation of desmin and troponin-T in type II fibres isolated from the five muscles is illustrated in figure 3. To the authors knowledge, the fibre type results presented in this study are the first to demonstrate postmortem changes specifically on type II muscle fibres. In this part of the study, degradation products of these proteins could not be detected. We speculate that this might be caused by diffusion of the degraded proteins into the bathing media upon dissection of the single fibres. The relative change in the band intensity of intact troponin-T and desmin, from day 1 to 8 p.m. was therefore calculated and used as an estimate of the rate of proteolytic degradation. Our results suggest that the highest relative change of desmin occurs in type II fibres isolated from LD and that desmin does not degrade from day 1 to 8 p.m. in ST. These results are in agreement with the proteolytic changes observed in whole muscle homogenates. VI exhibits the highest relative change of troponin-T from day 1 to 8 p.m., followed by LD and S, whereas no change of troponin-T was detected in ST and SM. This finding is not in agreement with the proteolytic changes of the muscle homogenates. It is not clear why the changes in the native troponin-T bands in type II fibres does not correspond to the findings in muscle homogenates. Arguably, the most important result from analysis of proteolysis in a single fibre type is that type II fibres show different patterns of proteolysis depending on the muscle in which they are located. This implies that proteolysis is not simply defined within a given fibre type, but that it is modulated by the local environment within each muscle.

Conclusions

Differences between muscles in the rate of proteolytic degradation do not seem to be a direct result of the fibre type distribution. The rate of postmortem proteolysis probably depends more on the local environment (i.e., pH and proteolytic potential) within the muscle as a whole rather than on variations between individual fibres of given types within the muscle.

Pertinent literature

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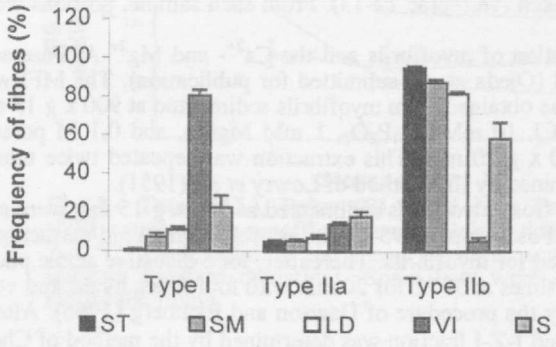

Figure 1. Percentage of type I, IIb and IIa fibres in five porcine muscles. Values are given as means ± S.E. (n=7)

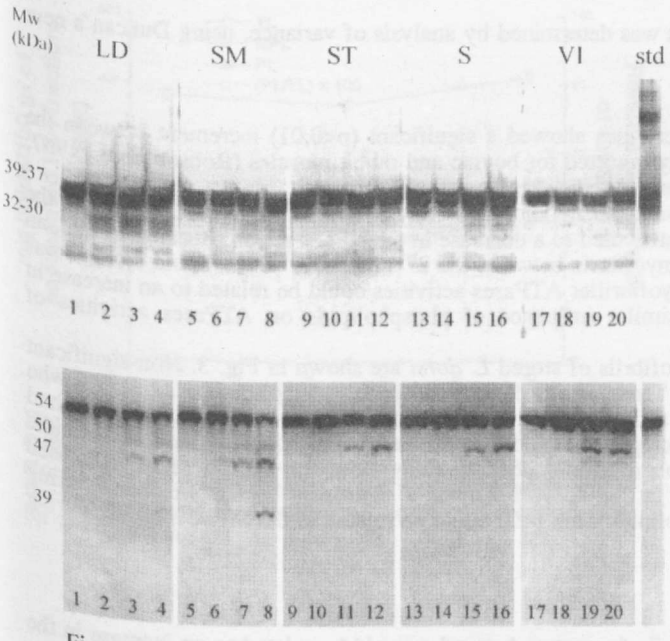

Figure 2. Western blot of crude muscle homogenates from the five different muscles at 0 (lane 1, 5, 9, 13, 17), 1 (lane 2, 6, 10, 14, 18), 3 (lane 3, 7, 11, 15, 19) and 8 (lane 4, 8, 12, 16, 20) days postmortem. (a) troponin-T and (b) desmin. Standards appear in the single track on the right. Each muscle was analysed from three different animals.

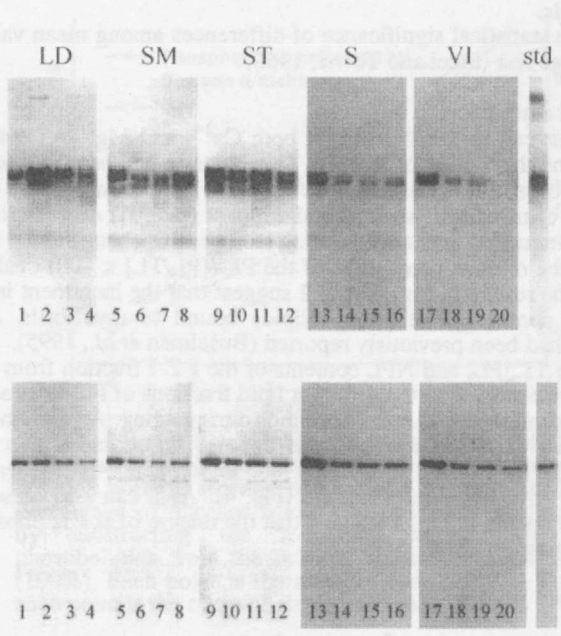

Figure 3. Western blot of type II muscle fibres isolated from the five different muscles at 0 (lane 1, 5, 9, 13, 17), 1 (lane 2, 6, 10, 14, 18), 3 (lane 3, 7, 11, 15, 19) and 8 (lane 4, 8, 12, 16, 20) days postmortem (a) troponin-T and (b) desmin. Standards appear in the single track on the right. Each muscle was analysed from one animal.