EFFECTS OF ELECTRICAL STIMULATION ON POST-MORTEM CHANGES IN MYOFIBRILLAR AND SARCOPLASMIC PROTEINS OF BEEF

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SUMMARY

Sixteen Holstein steers and cows were electrically stimulated at low voltage (40 V and 13.8 Hz) for 0, 30, 60 or 90 sec. Myofibrillar and sarcoplasmic proteins from *biceps femoris* stored at lflOC for 21 days were analyzed by SDS-PAGE and HPLC. The degradation of troponin T and production of 30 K component were observed in myofibrillar proteins of electrical stimulation (ES) muscles for 60 and 90 sec at the early stage (0-3 days). On the other hand, in the case of Holstein cows,the degradation of troponin T and production of 30 K component were observed in control and ES muscles at 14 days. However, there were a few bands between tropomyosin and 30 K component in ES muscles. Although sarcoplasmic proteins were not changed in the SDS-PAGE patterns until 7 days, the degradation ot sarcoplasmic proteins was observed at 0 day on the HPLC pattern in ES muscles.

INTRODUCTION

Many studies have been carried out on the changes of myofibrillar proteins during post-mortem storage, and these alterations have been related to the development in meat tenderness. Electrical stimulations (ES) has been recognized as a means of improving tenderness (Carse 1973; Salm et al. 1983). ES induces a rapid exhaustion of ATP and reduction in muscle pH, with a concomitant increase in muscle lactate and fall in glycogen. So this process hastens the time of rigor mortis and avoidance of cold shortening, because the meat achieves rigor before entry to a refrigerator or freezer (Bendall 1980). ES also causes disruption of the myofibrillar structure (Takahashi et al. 1987) and increases the activity of proteolytic enzymes (Dutson et al. 1980). Other muscle proteolytic enzymes could be involved in the conditioning process, as well the calcium-activated neutral protease (Ca ANP; Olson et al. 1977) and the lysosomal proteases (Ouali et al. 1987). There is also the possibility of a cooperative mechanism between Ca ANP and lysosomal proteases (Penny and Ferguson-Pryce 1979; Ouali and Valin 1981). Although Ca ANPs that have activity against myofibrillar proteins function around neutral pH, the pH of carcass rapidly falls bellow pH 6.0 after ES. So it is assumed that the activity of this protease is restricted in ES meat around ultimate pH. Therefore, the possibility for increased tenderness of ES meat could be due to the increased rate of enzymes release from the lysosomes into the rapidly acidifying environment within the muscle fiber and to the greater activity of these enzymes at low pH. Other merits of ES are the bright red color appearance of the meat and improvement in palatability etc. Our purpose is to confirm the proteolysis of myofibrillar and sarcoplasmic proteins in ES muscles from Holstein steers and cows under Japanese fattening conditions.

MATERIALS AND METHODS

Experiment I:

Eight Holstein steers, 18 month-old and 700 Kg liveweight, were slaughtered. ES of low voltage (40V, 13.8 Hz) was carried out on pair of steer for 30, 60 and 90 sec, respectively within 5 min after slaughter. Two steers were used as controls. At 3hr post-mortem, samples of *biceps femoris* muscles were obtained and divided into 6 portions of approximately 400 g. Each muscle was packed into a Cryovac bag and stored at 1 ± 1°C within 3 or 4hr after slaughter. Muscles were then analyzed at 0, 3, 5, 7, 14 and 21 days.

Experiment II:

Eight Holstein cows, 6-7 year-old and 750-800 Kg liveweight, were slaughtered. Low voltage ES was carried out for 60 sec for 5 cows within 5 min after slaughter. Three cows were used as controls. Muscle preparation and storage were the same as in Experiment I. After storage, the bags were opened, and 10 g of muscles was homogenized with 30 ml of rigor buffer in an ice water bath, centrifuged at 1°C, 11,000 g for 20 min. The supernatants were filtered through Toyo No.5c filter paper and then passed through a 0.2 μm membrane filter. This was used as sarcoplasmic proteins for the sample of SDS polyacrylamide gel electrophoresis (SDS-PAGE) and high performance liquid chromatography (HPLC). Myofibrils were prepared from the centrifugation of the rigor buffer homogenate, repeating the homogenization with rigor buffer and centrifugation. SDS-PAGE of the myofibrillar and sarcoplasmic proteins was performed according to the method of Laemmli (1970) and a 50 μg sample of protein was loaded into each slot. For HPLC a model CCP-8000 system (Toso Co.) was used. In this study the column of TSK-G3OOOSW (d.7x60 cm) and G2OOOSW (0.7X60 cm) were connected. The elution buffer was 0.25 M phosphate pH 6.5 and pumped at a flow rate of 0.5 ml/min. Sample injection was at a constant volume of 10 μl, and proteins were detected by absorbance at 280 nm.

RESULTS

SDS-PAGE patterns of myofibrillar proteins from Holstein steers are showed in Fig.1. The degradation of troponin T and the concomitant appearance of a 30 K component in ES muscles were observed at an earlier stage compared with the control. This finding was most apparent in ES 60 and 90 sec muscles. During conditioning of meat from these two groups, troponin T

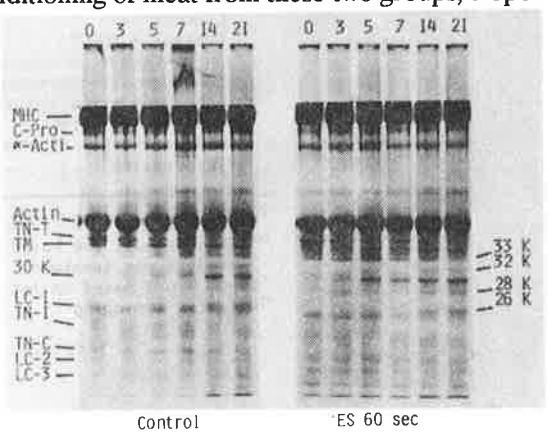

Fig. 1. SDS-PAGE patterns of myofibrillar proteins from Holstein steers. The numbers of 0 to 21 gives the storage time in days at 1°C after slaughter. Electrophoresis was performed in a 11% polyacrylamide slab gel.

was degraded by 5 days and the 30 K component had appeared at 0 and 3 days, respectively. Besides new bands (33 K component) had appeared below the tropomyosin band. In the case of Holstein cows, troponin T was degraded and the 30 K component had appeared at 14 days in control. In the case of the 33 K component, this had appeared at 0 day in ES 60 sec muscles and at 14 days in control. Tropomyosin was slightly degraded both in control and ES muscles by 21 days (Fig.2). Other changes found in the myofibrillar proteins patterns during post-mortem storage were the appearance of 26, 28 and 32 K components and these bands were most noticeable in ES muscles. SDS-PAGE patterns of sarcoplasmic proteins from Holstein steers and cows showed the degradation of 33, 36 and 44 K components in control and

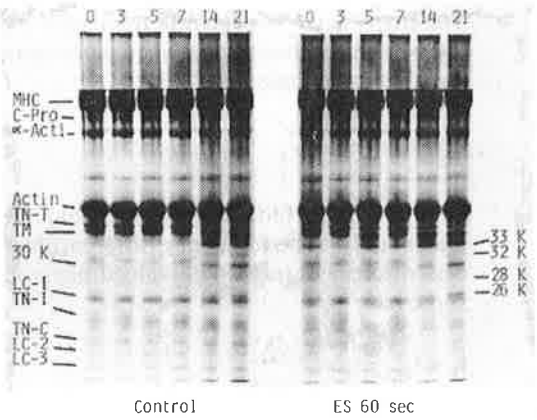

Fig. 2. SDS-PAGE patterns of myofibrillar proteins from Holstein cows. The numbers and electrophoretic conditions are the same as in Fig.1.

ES muscles at 7 and 14 days, although there were some changes to be seen in the HPLC patterns at 0 day.

From the analysis of sarcoplasmic proteins using HPLC, we can observe changes in the peaks of proteins, peptides and amino acids. After ES treatment, some protein components were degraded as in the peaks of RT-61 and 65, and new peaks appeared as at RT-58, 70 and 77. The peak of RT-81 was mainly myoglobin and became larger after ES treatment. The peaks from RT-93 to 103 were peptides and amino acids, and some of these peaks were degraded or replaced by new peaks (RT-103; Fig. 3). From these facts it was found that ES caused the degradation of sarcoplasmic proteins and made some peptides and amino acid concerning the palatability.

DISCUSSION

Temperature and pH are well established as important factors in post-mortem muscle tenderization (Yates et al. 1983), In the environment of high temperature and low pH, it is assumed that these are favorable condition for acidic proteases such as cathepsins B and L, because ES treatment causes a rapid fall in muscle pH and a more rapid release of lysosomal enzymes (Moeller et al. 1976 and 1977; Dutson et al. 1980). The apparent changes of myofibrillar proteins in ES muscles are the production of the components of 26, 28, 30, and 33 K. Ducastaing et al. (1985) also reports the appearance of 30 and 32 K components at 4hr post-mortem after ES treatment. Cathepsin B and L degrade myosin and other myofibrillar proteins (Noda et al. 1981; Mikami et al. 1988). However,

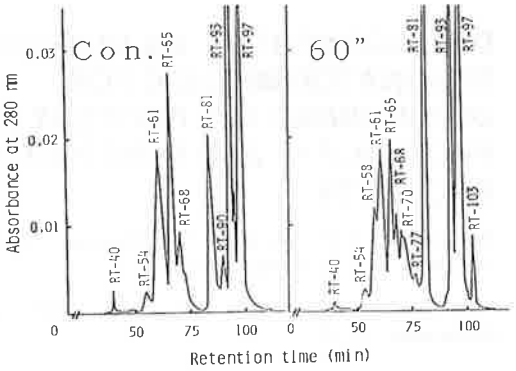

Fig. 3. HPLC chromatogram of sarcoplasmic proteins from Holstein steer. Column, TSK-G3000SW G2000SW; buffer, 0.25 M phosphate pH 6.5; flow rate, 0.5 ml/min; sample, sarcoplasmic proteins at 0 day after slaughter; Con, control; 60", ES 60 sec.

degradation of myosin, α−actinin and actin was not clearly identified from the SDS-PAGE patterns. The main reason was probably due to the low temperature (1±1°C) of conditioning in this experiment. However, this temperature is normal employed in meat storage. We can not certain thous which proteases had contributed to conditioning in ES meats, although myofibrillar and sarcoplasmic proteins were degraded faster in ES muscles compared with control.

CONCLUSION

Myofibrillar proteins from Holstein steers were degraded and produced several bands in ES muscles on SDS-PAGE patterns. However, the degradation of myofibrillar proteins from Holstein cows was slower than that of steers in control and ES muscles. It was found from the HPLC patterns that sarcoplasmic proteins were degraded and produced several new peaks of these fragments at 0 day in ES muscles.

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