THE CALPAIN SYSTEM IN CHICKEN SKELETAL MUSCLE AND CHANGES IN PROTEOLYTIC ACTIVITY DURING AGEING

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Introduction

Post mortem tenderisation of meat depends largely on the action of proteases, which weaken myofibrillar structures. Among these proteases, calpains probably constitute the proteolytic system that plays a key role in meat ageing, at least in the first few hours. The role of calpains in *post mortem* proteolysis and tenderisation is well documented, particularly in cattle (Koohmaraie, 1996; Geesink *et al.*, 2000), where μ-calpain is the active form able *in vitro* to reproduce observations made *in vivo*, unlike m-calpain, which requires high calcium concentrations. However, as shown by Boehm *et al.*, (1998) in cattle and Veiseth *et al.*, (2001) in sheep, μ-calpain activity drops very quickly after death and probably cannot account for all the phenomena observed. In cattle, the proteasome may also participate in tenderisation (Robert *et al.*, 1999) as it damages myofibrils *in vitro* and remains active for eight days after slaughter (Lamare *et al.*, 2000). Data on meat ageing are incomplete and contradictory in chickens (Shreurs, 2000), in which 80% of maximum tenderness is reached within 10 hours after slaughter, compared with 10 days in cattle (Dransfield, 1994). The nature of calpains in chicken is unclear, and one, two or even three ubiquitous calpains of differing calcium sensitivity have been reported (Ishiura *et al.*, 1978; Wolfe *et al.*, 1989; Sorimachi *et al.*, 1995). Our aim was to identify the special features of calpains in chicken, in comparison with calpains in cattle, and to study *post mortem* changes in the calpain system.

Materials and Methods

Samples of about 2 grams of *Pectoralis superficialis* muscle were collected at different times (5 min, 1 h, 3 h, 6 h, 12 h, 24 h, 48 h, 72 h) after death, from nine 7-week-old female chickens whose carcasses were kept at 4°C in the slaughterhouse of the Theix INRA research centre. Samples were frozen in liquid nitrogen, powdered and stored at -80°C until use. Calpain activity was measured by zymography. Samples were analysed by non-denaturing electrophoresis in gels copolymerised in the presence of casein, EDTA and EGTA, according to a slightly modified version of the method of Raser *et al.*, (1995). Gels were incubated in the presence of calcium and a reducing agent that activates calpains. In the region of a band of activated calpain, the casein was digested into small fragments which diffused out of the gel. The gels were then stained with Coomassie blue, and a clear band was noted in the presence of calpain. The bands were digitised using a scanner (Umax) and Photoshop software. The signals obtained were quantified using QuantityOne software (Bio-Rad). Under our experimental conditions, the signal was proportional to the amount of protein loaded on the gel.

Results and Discussion

Specificity of chicken calpains: Calpain activities were measured in raw muscle extracts from cattle and chicken. Zymograms were incubated in the presence of different calcium concentrations. Figure 1 shows that four types of calpains appeared successively. In the absence of calcium, no activity was visible, thus demonstrating the method's specificity. In the presence of 10 μM calcium, a calpain of low mobility was seen in chicken (1), and bovine μ-calpain appeared at 30 μM calcium (2). The second form in chicken was seen in the presence of low calcium concentration (100 μM calcium) (3) whereas in bovine, the m-calpain was activated at 1 mM and 5 mM calcium (4). In addition, the proportions of μ- and m-calpains differed greatly, since in cattle they were present in roughly equal quantities, whereas in chicken μ-calpain was found in small amounts and accounted for under 15% of total calpain activity.

Post mortem changes in calpain activity in chicken muscle: Calpain activity was measured by zymography using chicken muscle extracts obtained at different times *post mortem* (see Materials and Methods). μ-calpain activity was very low at the outset and rapidly diminished until it was negligible 12 hours *post mortem* (Figure 2). μ/m-calpain activity was stable and from 24 hours onwards there was a slight decrease, which was less than 10% after 72 hours. A third form of calcium-dependent protease appeared after 6 hours and progressively increased up to 72 hours. Preliminary work suggests that this was a phosphorylated form of μ/m-calpain.

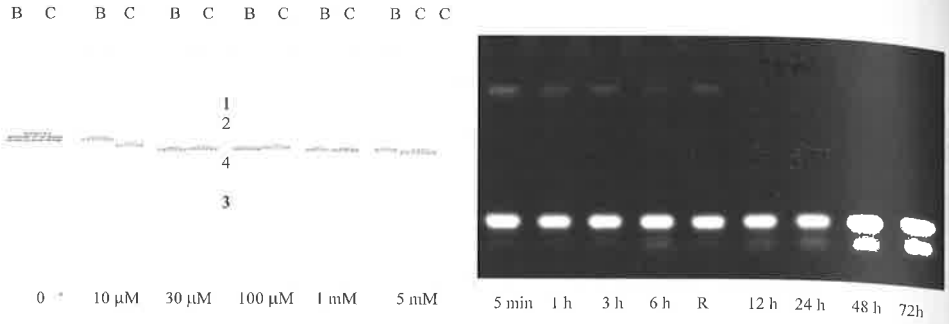

Figure 1: Activities of calpains from bovine *semi Tendinosus* muscle and/or chicken muscle *pectoralis superficialis* measured by zymography, as a function of calcium concentration B = bovine; C = chicken.

Figure 2: *Post-mortem* changes in calpain activities in chicken *pectoralis superficialis* muscle. R= reference.

Chicken and mammalian calpain isoforms differ in electrophoretic mobility and the calcium sensitivity of the former is much greater than that of the latter, justifying the name µ/m-calpain for the form with the greatest electrophoretic mobility. In contrast to the two mammalian forms, which are present in roughly equivalent amounts, in chicken the µ/m-calpain form predominates and µ-calpain accounts for less than 15% of the total activity. The calcium sensitivity of these chicken calpains means that they are active soon after death, and µ-calpain activity decreases sharply in the first few hours (and fit the pH decline in muscle) due to autolysis. It is possible that, as in mammals, chicken µ-calpain is in the active form *post mortem* and causes early destabilisation of myofibrillar structures, thereby permitting the action of other proteolytic systems like µ/m-calpain or the proteasome. Tenderisation of chicken muscle does indeed take place in the first few hours *post mortem* (Dransfield, 1994). Moreover, we have shown in the laboratory that 50% of proteasome activity in chicken muscle remains 48 hours after slaughter.

References

Boehm, M.L., Kendall T.L., Thompson V.F. and Goll D.E. (1998). Changes in the calpains and calpastatin during post-mortem storage of bovine muscle. *Journal of Animal Science,* 76, 2415-34.

Dransfield, E. (1994). Optimisation of tenderisation, ageing, and tenderness. *Meat Science,* 36, 105–121.

Geesink G.H., Ilian M.A., Morton J.D. and Bickerstaffel R. (2000). Involvement of calpains in postmortem tenderization. In *Proceedings of the New Zealand Society of Animal Production*, 60, 99-102.

Ishiura S., Murofushi H., Suzuki K. and Imahori K. (1978). Studies of a calcium-activated neutral protease from chicken skeletal muscle. I. Purification and characterization. *J. Biochem. (Tokyo),* 84, 225-30.

Koohmaraie M. (1996) Biochemical factors regulating the toughening and tenderization process of meat. *Meat Science,* 43, 193-201.

Lamare M.C., Taylor R.G., Farout L., Briand Y. and Briand M. (2002). Changes in proteasome activity during postmortem aging of bovine muscle. *Meat Science.,* 61, 199-204.

Raser K.J., Posner A. and Wang K.K. (1995). Casein zymography: a method to study m-calpain, m-calpain, and their inhibitory agents. *Archives Biochemistry and Biophysics,* 319, 211-6.

Robert N., Briand M., Taylor R.G., Briand Y. (1999). The effect of proteasome on myofibrillar strucutures in bovine skeletal muscle. *Meat Science,* 51, 149-53.

Schreurs F.J.G. (2000). Post-mortem changes in chicken muscle. *World's Poultry Science Journal,* 56, 319-46.

Sorimachi H., Tsukahara T., Okada-Ban M., Sugita H., Ishiura S. and Suzuki K. (1995). Identification of a third ubiquitous calpain species: chicken muscle expresses four distinct calpains. *Biochimica Biophysica Acta.,* 1261, 381-93

Suzuki A. and Sorimachi H. (1998). A novel aspect of calpain activation. *FEBS Letters,* 433(1-2), 1-4.

Veiseth E., Shackelford S.D., Wheeler T.L. and Koohmaraie M. (2001). Effect of postmortem storage on mu-calpain and m-calpain in ovine skeletal muscle. *Journal of Animal Science,* 79(6), 1502-8.

Wolfe F.H., Sathe S.K., Goll D.E., Kleese W.C., Edmuns T. and Duperret S.M. (1989). Chicken skeletal muscle has three Ca2+-dependent proteinases. *Biochim. Biophys. Acta.,* 998, 236-50.