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Influence of caspase3 selective inhibitor on proteolysis of chicken skeletal muscle proteins during *post mortem* aging

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ABSTRACT

The objective of this study was to investigate the potential contribution of apoptosis related downstream executioner caspase3 to *post mortem* skeletal muscle proteolysis by use of caspase3 selective inhibitor DEVD–CHO (*N*-acetyl–Asp–Glu–Val–Asp–CHO). After slaughter, four chicken breast muscles were removed and cut into small pieces, then marinated in treatment solution containing DEVD–CHO, or in control solution, and stored at 4 °C for 1, 3 or 7 d. Meat samples were obtained and used for detecting muscle protein degradation or calpain activity. Results showed that DEVD–CHO had inhibited the degradation of muscle skeletal proteins (titin, nebulin, desmin and troponin-T) significantly, whereas the activity of calpains had not been influenced. Therefore, the degradation of muscle proteins should not been exclusively attributed to the calpain system, and the effector caspase3 may be a new protease involved in meat *post mortem* tenderization.

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1. Introduction

Meat palatability is mainly determined by tenderness, flavour and juiciness. However, consumers consider tenderness as the most important eating quality characteristic of meat (Shackelford et al., 2001). In addition, variability in the toughness of both beef and lamb has been highlighted by retail survey (Brooks et al., 2000). In practice, storage of meat, especially beef, lamb and pork, for a given time, is a prerequisite for the development of tenderness. It has been well established that *post mortem* limited degradation of myofibrillar proteins has a close relationship to the structural destruction and improved tenderness of meat during refrigerated storage (Huff-Lonergan et al., 1996a; Koohmaraie, 1996; Taylor, Geesink, Thompson, Koohmaraie, & Goll, 1995).

Actually, the meat tenderizing process is generally assumed to be enzymatic in nature, although there is some dispute (Geesink, Taylor, Bekhit, & Bickerstaffe, 2001). Generally, four major proteolytic systems occur in muscles. The lysosomal and proteasomal systems can operate an exhaustive degradation of cell proteins, into amino acids or small peptides, while, due to restricted specificity, calpain and caspase systems cause a limited proteolysis (Costelli et al., 2005). Accumulated evidence suggests that lysosomal cathepsins and proteasome may make little, if any, contribution to meat protein limited degradation and, as such, meat tenderization (Baron, Jacobsen, & Purslow, 2004; Delgado, Geesink, Marchello, Goll, & Koohmaraie, 2001; Ouali et al., 2006). Among the most studied proteolytic systems, the roles of calpains, especially, μ-calpain, are most highlighted in meat tenderization. However, they cannot explain the major changes occurring in meat aging because, even in the presence of effective synthetic and natural calpain inhibitors, myofibrillar proteins are still cleaved (Herrera-Mendez, Becila, Boudjellal, & Ouali, 2006; Hopkins & Thompson, 2002). Hence, it is possible that, in addition to the calpains and proteases mentioned above, other proteases may be acting and are possibly involved in meat *post mortem* aging.

The term of apoptosis was first introduced in 1972 by John Kerr to designate the common morphological features of programmed cell death in tissue remodelling, and it was studied extensively thereafter. The process and its molecular components are conserved through virtually all metazoan species studied so far (Huppertz, Frank, & Kaufmann, 1999). Cell death through apoptosis has two major pathways (of extrinsic and intrinsic origin), both of which lead equally to the activation of death executioner proteases, consisting of caspases 3, 6, and 7, which, (especially caspase3), are responsible for most of the cleavage events observed during apoptosis (Adams, 2003; Gross, McDonnell, & Korsmeyer, 1999). After animal bleeding, all of the muscle fibre will be irreversibly deprived of nutrients and oxygen and have no alternative but engage towards suicide (Brunelle & Chandel, 2002). Consequently, it is conceivable that caspases could play roles in *post mortem* aging.

Currently, little is known about the role of effective caspases in meat *post mortem* tenderization. The objectives of this study were to test the hypothesis that caspase3 is involved in the proteolysis of muscle proteins. To this end, we compared the protein

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degradation patterns between selective caspase3 inhibitor-treated and control samples during *post mortem* aging.

2. Materials and methods

2.1. Chickens and treatment

Four layer chickens (six months, approximately 2.5 kg), from the animal experimental station attached to Nanjing Agricultural University, were cared for and slaughtered as outlined in the guide for the care and use of experimental animals (Animal Experimental Special Committee of NAU). After being sacrificed, the four chicken breast muscles (*Musculus pectoralis superficialis*) were rapidly removed and dissected into small pieces (ca. 0.5 g/piece). All birds were slaughtered and treated individually on the same day. The 0 d sample was obtained within 10 min, after the chicken was slaughtered, and snap-frozen in liquid nitrogen, and then stored at −80 °C prior to subsequent analysis. Other samples were divided into two portions and soaked in treatment solution containing 100 μM DEVD–CHO (BIOMOL, International, LP), 100 mM NaCl and 2 mM NaN_3 or control solution (same as treatment solution with DEVD–CHO omitted) in the ratio 1:2 (w/v) (meat: solution), and stored at 4 °C for 1, 3 or 7 d. At each time point, meat samples were obtained and treated as were 0 d samples.

2.2. Sarcoplasmic protein extraction

Sarcoplasmic proteins were extracted according to the procedure reported. (Veiseth, Shackelford, Wheeler, & Koohmaraie, 2001) with modifications, as noted in the following description. Finely minced meat samples (0.3 g) were homogenized with polytron at a speed of 15,000 rpm for 15 s, two times, with a 15 s interval cooling period between bursts; 0.3 g of chicken breast muscle was homogenized in 5 volumes (w/v) of extraction buffer containing 10 mM EDTA, 0.1% (v/v) β-mercaptoethanol (MCE), protease inhibitor cocktail (10 ml/tablet; Roche applied science, Mannheim Germany) and 100 mM Tris HCl, pH 8.3. The homogenate was centrifuged at 15,000 *g* for 30 min at 4 °C, and then the supernatant was collected and used for determination of the relative calpain activity with casein zymography. Protein content was determined with the BCA Protein Assay Kit, following the standard protocol recommended by the manufacturer (Pierce).

2.3. Myofibril preparation

Myofibrils from 0 d and *post mortem* muscle tissues were purified at 2 °C according to the myofibril preparation procedure with small modifications (Etlinger, Zak, & Fischman, 1976). The minced muscle was homogenized in 7.5 volumes of PRB buffer (100 mM KCl, 2 mM $MgCl_2$, 2 mM EGTA, 1 mM DTT, 1 mM NaN_3, 2 mM $Na_4P_2O_7$, 10 mM Tris-maleate, pH 6.8) with a blade type homogenizer (VirTis) at a speed of 10,000 rpm for 60 s, and then the homogenate was centrifuged at 1000*g* for 10 min. The pellet was subsequently washed eight times, each wash with 10 volumes of low salt buffer (same as PRB except for pyrophosphate being omitted). In the last step, the myofibrils were suspended in Tris–EDTA buffer (10 mM Tris, 5 mM EDTA, pH 8.0) and immediately removed and mixed with treatment buffer (125 mM Tris, 4% SDS, 20% glycerol); the samples were heated in a 50 °C water bath for 20 min, and then centrifuged for 30 min (16,000 *g*). Protein concentration was determined with the BCA Protein Assay Kit, and diluted to 3.5 mg/ml using treatment buffer containing 10% MCE and 0.001% bromophenol blue. Samples were well mixed and heated at 50 °C for 10 min, and then stored at −80 °C for subsequent SDS–PAGE and western blotting.

2.4. Casein zymography

The previously reported casein zymography procedure was followed, with slight modification (Melody et al., 2004). One volume of supernatant (from the sarcoplasmic fraction) was combined with 1 volume of tracking dye solution (20% [v/v] glycerol, 0.75% [v/v] MCE, 0.05% [w/v] bromophenol blue and 150 mM Tris–HCl, pH 6.8) and then stored at −80 °C for subsequent analysis. Samples were thawed and loaded immediately onto nondenaturing 12.5% PAGE casein gels (separating gel = acrylamide:*N*,*N*′-bis-methylene acrylamide = 70:1 [w/w], 0.05% [v/v] *N*′*N*′*N*′*N*′-tetramethylethylenediamine [TEMED], 0.05% [w/v] ammonium persulfate [APS], casein [2.1 mg/ml], and 375 mM Tris_HCl, pH8.8; stacking gel = acrylamide:*N*,*N*′-bis-methylene acrylamide = 37.5:1 [w/w], 0.125% [v/v] TEMED, 0.075% [w/v] APS, and 125 mM Tris_HCl, pH 6.8). Gels (9 cm wide, 8 cm tall) were run on mini slab electrophoresis units (Bio-Rad Laboratories, Hercules, CA). Gels were pre-run at 100 V for 15 min. The running buffer contained 192 mM glycine, 1 mM EDTA, 0.05% (v/v) MCE, and 25 mM Tris, pH 8.3. Gels were run at a constant voltage (100 V) for approximately 10 h, and then incubated in three changes (20 min each) of 5 mM $CaCl_2$, 0.1% (v/v) MCE, and 50 mM Tris_HCl, pH 7.5. Gels were then incubated overnight in 5 mM $CaCl_2$, 0.1% (v/v) MCE, and 50 mM Tris_HCl, pH 7.5. The following day, gels were stained in a solution containing 0.1% (w/v) Coomassie brilliant blue R-250, 40% (v/v) methanol, and 7% (v/v) glacial acetic acid for approximately 2 h, and subsequently were destained using an excess of destain (40% [v/v] methanol and 7% [v/v] glacial acetic acid). Calpain activity was indicated by clear zones in the stained gels.

2.5. Gel system

Ten percent and 12.5% polyacrylamide separating gels (acrylamide:*N*,*N*′-bis-methylene acrylamide = 37.5:1 [w/w],0.1% [w/v] SDS, 0.05% [v/v] TEMED, 0.05% [w/v] APS, and 0.5 M Tris_HCl, pH 8.8) were used to detect the myofibril proteins changes of desmin and troponin-T, respectively. A 5% polyacrylamide gel (acrylamide:*N*,*N*′-bis-methelyene acrylamide = 37.5:1 [w/w], 0.1% [w/v] SDS, 0.125% [v/v] TEMED, 0.075% [w/v] APS, and 0.125 M Tris_HCl, pH 6.8) was used for the stacking gel. A 5% polyacrylamide continuous gel (acrylamide:*N*,*N*′-bis-methylene acrylamide = 100:1 [w/w], 0.1% [w/v] SDS, 0.067% TEMED, 0.1% [w/v] APS, 2 mM EDTA, and 200 mM Tris_HCl, pH 8.0) was used for determination of titin and nebulin (Huff-Lonergan, Mitsuhashi, Parrish, & Robson, 1996b).

2.6. SDS–PAGE

Gels (9 cm wide × 8 cm tall) for analysis of desmin degradation were run on AE-6530M mini slab electrophoresis units (Bio-Rad Laboratories, Hercules, CA). The running buffer contained 25 mM Tris, 192 mM glycine, and 0.1% [w/v] SDS. Gels were loaded with 40 μg per well of protein for desmin, 20 μg for troponin-T, and run at a constant voltage of 200 V for approximately 1.2 h. For 5% gels, 80 μg of myofibril samples were loaded. Gels (8 cm wide × 7.3 cm tall × 1.5 mm thick) were run on the Bio-Rad Mini-Protean II system (Bio-Rad Laboratories, Hercules, CA). The gels were run at a constant current setting of 5 mA/gel for 18 h. For examination of all protein bands, gels were stained at a minimum of 12 h in an excess of 0.1% (w/v) Coomassie brilliant blue R-250, 40% (v/v) ethanol, and 7% (v/v) glacial acetic acid. Gels were destained in an excess of the same solution without the Coomassie brilliant blue R-250.

2.7. Western blotting

Gels for desmin were immediately transferred to nitrocellulose membranes (Bio-Rad Laboratories, CA) using a Mini-Protean II system (Bio-Rad Laboratories, Hercules, CA) at a constant current of 200 mA for 1 h at 4 °C. The transfer buffer consisted of 25 mM Tris, 192 mM glycine, and 10% (v/v) methanol. The electroblotted membrane was then blocked overnight in blocking buffer (TTBS: 0.05% Tween 20, 20 mM Tris, 137 mM NaCl, 5 mM KCl and 5% skim milk powder) at 4 °C. After blocking, the membrane was incubated for 1 h at room temperature with polyclonal rabbit antibody raised against chicken skeletal muscle protein desmin at a dilution of 1:1000 in blocking buffer; the antibody was prepared in the meat laboratory, Hokkaido University, and identified as only one immunoreactive band observed by western blotting (data not shown) and generously gifted. After four washes with TTBS for 5 min each, the membrane was incubated with goat anti rabbit IgG horseradish peroxidase, conjugated affinity purified second antibody (Chemicon) at a dilution of 1:10,000 in blocking buffer. After four more washes, the membrane was stained with a Sigma fast 3, 3′-diaminobenzidine tablet set (Sigma–Aldrich, St. Louis, USA).

2.8. Semi quantification of targetted proteins

The gels or membranes were scanned (with a scanner, GT-800F EPSON) at a resolution of 600 dpi, and then the densities of bands were quantified by image J (1.37v/ Java). Densitometric analyses were performed using Scion Image software (Scion Corporation, Frederick, MD, USA). All densitometric scans of gels and Western blotting bands were within the calibration range. Troponin-T degradation was indicated by an increase of the 30 kDa band; desmin degradation was indicated by a decrease in intensity of an approximately 55 kDa band. Intact desmin degradation ratio was calculated as the intensity of each immunoreactive desmin band over the intensity of the immunoreactive desmin band in a reference sample (at-death) that was loaded onto each gel. Troponin-T degradation product ratios were calculated as the intensity of the30 kDa fragment band in each gel over that of the actin within the same lane.

2.9. Statistical analyses

The data from four replicates were analysed by one-way ANOVA using the SPSS statistical package programme, and differences among the individual means were compared using Duncan's multiple range test (SPSS, 1998), effects were considered significant at $p < 0.05$.

3. Results

3.1. Protein degradation

3.1.1. General

As expected, there were great differences in muscle protein proteolysis between muscle samples of DEVD–CHO-treated and control during *post mortem* conditioning, evidenced by SDS–PAGE or Western blotting, with the protein degradation in the former being consistently at a slower rate.

3.1.2. Nebulin and titin

The *post mortem* degradation patterns of giant myofibrillar proteins titin and nebulin were followed using 5% continuous SDS–PAGE gels for up to 7 d at 4 °C. Results showed that the degradation of nebulin was effectively inhibited by the selective caspase 3 inhibitor DEVD–CHO (Figs. 1 and 2) although the intact nebulin

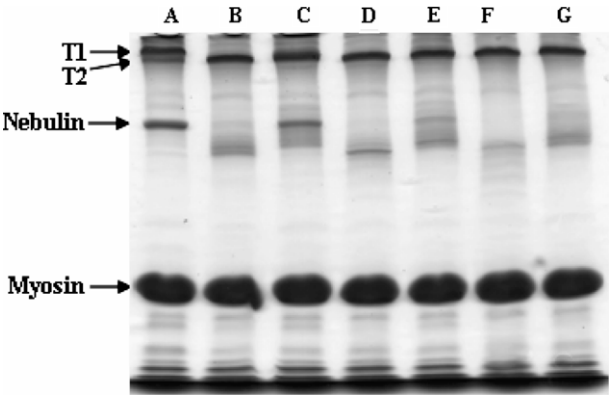

Fig. 1. Representative electrophoresis patterns of Coomassie-stained 5% polyacrylamide gel depicting titin and nebulin degradation after 0, 1, 3, and 7 days of muscle incubation at 4 °C in solutions containing 100 mM NaCl and 2 mM NaN_3, with or without caspase 3 inhibitor (DEVD–CHO). Lanes from left to right are: 0 d control (A), 1 d control (B), 1 d DEVD–CHO treatment (C), 3 d control (D), 3 d DEVD–CHO treatment (E), 7 d control (F), and 7 d DEVD–CHO treatment (G). Abbreviations are as follows: T1 = intact titin, T2 = large degradation product of intact titin; Myosin = myosin heavy chain. Eighty microgrammes of proteins were loaded per lane.

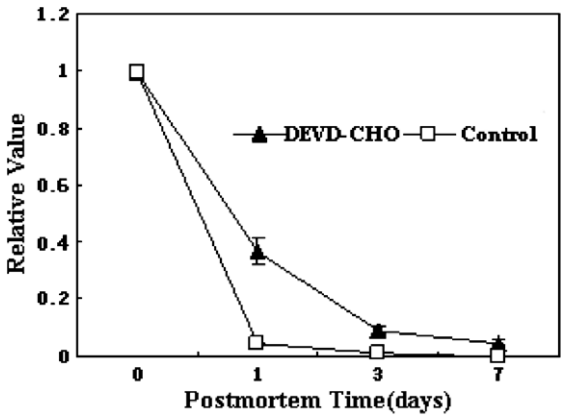

Fig. 2. Densitometric analysis of the degradation of nebulin during different incubation periods with DEVD–CHO (▲) or not (□). Ratios were calculated as intensity of the intact nebulin band in each gel over that of the 0 d intact nebulin band. Data are representative of four independent experiments and are presented as means ± standard deviation.

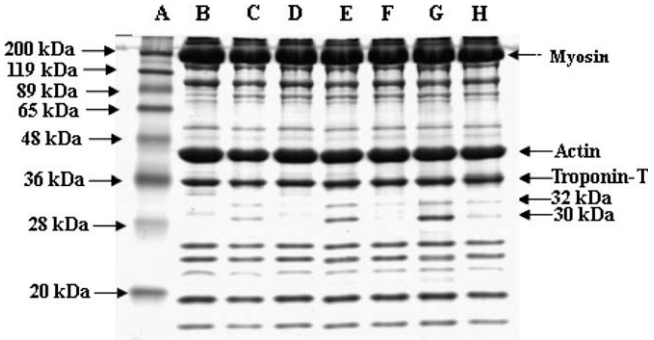

Fig. 3. Representative electrophoresis patterns of Coomassie-stained 12.5% polyacrylamide gel depicting the appearance of 32 and 30 kDa degradation fragments after 0 d, 1, 3, and 7 days of myofibril incubation at 4 °C in solutions containing 100 mM NaCl and 2 mM NaN_3, with or without caspase 3 inhibitor (DEVD–CHO). Lanes from left to right are: molecular standards (A), 0 d control (B), 1 d control (C), 1 d DEVD–CHO treatment (D), 3 d control (E), 3 d DEVD–CHO treatment (F), 7 d control (G), and 7 d DEVD–CHO treatment (H). Fifteen microgrammes of proteins were loaded per lane.

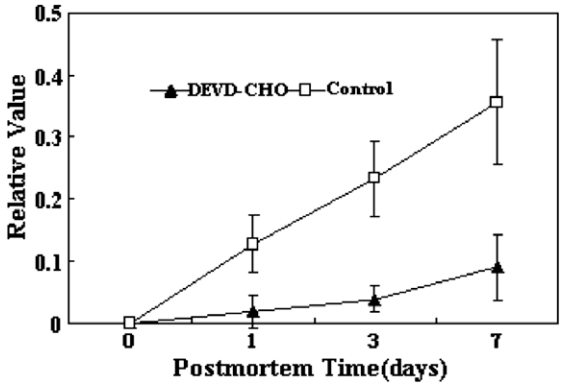

Fig. 4. Densitometric analysis of the appearance of the 30 kDa degradation fragment during different incubation periods with DEVD–CHO (▲) or not (□). Ratios were calculated as intensity of the 30 kDa fragment in each gel over that of the actin within the same lane. Data are representative of four independent experiments and are presented as means ± standard deviation.

bands intensity in all lanes declined progressively with time. After 1 d *post mortem*, approximately 95% of intact nebulin in control samples was degraded, and could almost not be visualized thereafter. Nevertheless, in DEVD–CHO-treated samples, about 37% of nebulin remained intact at 1 d *post mortem*, and the initial band of nebulin could be visualized, even after 7 d of *post mortem* storage. At all the aging time points, the nebulin degradation extents between treated and control were significantly different ($p < 0.05$). Titin degradation was not quantitatively analysed due to the difficulty in separating the native and degraded bands; however, the difference in titin proteolysis between the DEVD–CHO-treated and control samples could still be observed (Fig. 1), although the inhibition effect of DEVD–CHO on titin digestion was not as effective as on nebulin.

3.1.3. Troponin-T

The *post mortem* degradation of troponin-T and the appearance of bands ranging from approximately 28–32 kDa has frequently been reported (Ho, Stromer, & Robson, 1994; Huff-Lonergan et al., 1996a; Taylor et al., 1995). In the current study, the 12.5% SDS–PAGE gels also showed a difference between the DEVD–CHO and control samples in the appearance of 30 and 32 kDa degraded fragments (Fig. 3). At 1 d *post mortem*, in the naturally aging samples, the 30 kDa band could be readily detected, and the 32 kDa band, although not as apparent the 30 kDa, also appeared (Fig. 3). The temporal change of 30 kDa degradation products was evident. Densitometric analysis showed that, at 1, 3, 7 d *post mortem*, the control sample 30 kDa band intensities over that of actin within the same lane, amounted to 12.8%, 23%, 35%, respectively (Fig. 4). However, in treated samples, when compared to control, the rate of troponin-T degradation was reduced considerably. The 30 kDa band could not easily be observed until 7 d of aging, which only accounted for about 9% of that of actin. In all the experimental time points, the differences in the 30 kDa degraded fragments between the DEVD–CHO treated and control samples were significant ($p < 0.01$).

3.1.4. Desmin

Desmin degradation during *post mortem* aging was probed with a polyclonal antibody; Western blots results revealed that DEVD–CHO treatment had significant effects on desmin degradation (Fig. 5a). Overall, it was evident that the DEVD–CHO treated samples showed slower disappearance of intact desmin than did the control. At 1, 3, and 7 d *post mortem*, in control samples, approximately 57%, 90%, and 100% of intact desmin were degraded while, in DEVD–CHO-treated samples, approximately 69%, 55%, and 53% of desmin still existed in intact form at the three time points, respectively (Fig. 5b). During 7 d of aging, the difference in desmin

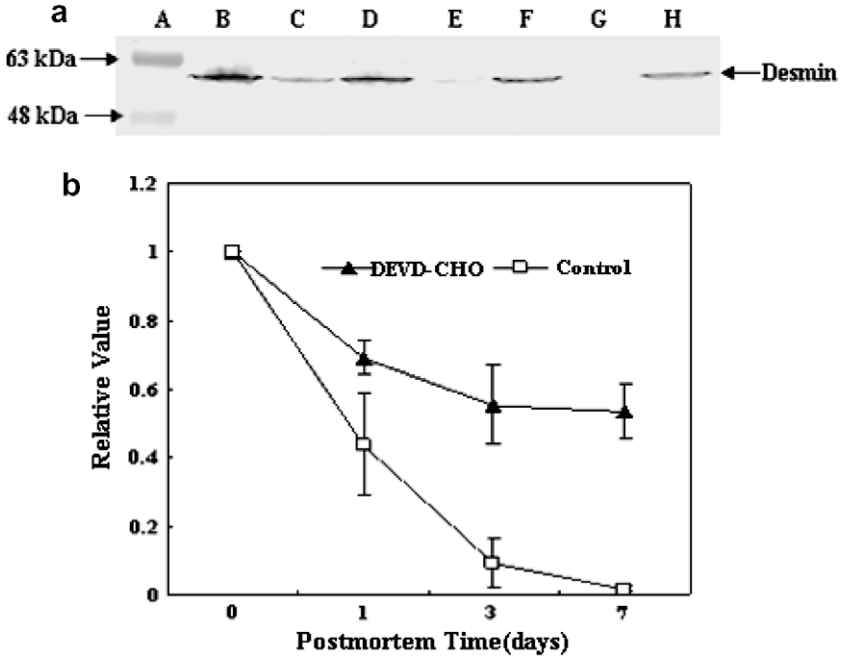

Fig. 5. Changes of desmin after 0, 1, 3, and 7 days of chicken breast muscle incubation at 4 °C in solutions containing 100 mM NaCl and 2 mM NaN_3, with or without caspase 3 inhibitor (DEVD–CHO). (a) Representative Western blotting pattern showing the degradation of desmin. Lanes from left to right are: molecular standards (A), 0 d control (B), 1 d control (C), 1 d DEVD–CHO treatment (D), 3 d control (E), 3 d DEVD–CHO treatment (F), 7 d control (G), and 7 d DEVD–CHO treatment (H). Forty microgrammes of proteins were loaded per lane. (b) Densitometric analysis of the degradation of desmin during incubation periods with DEVD–CHO (▲) or not (□). Ratios were calculated as the blot intensity of the desmin fragment in each gel over the intensity of desmin at 0 d. Data are representative of four independent experiments and are presented as means ± standard deviation.

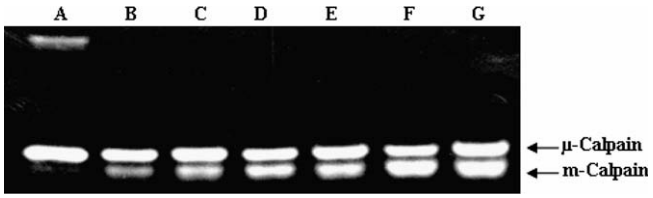

Fig. 6. Representative casein zymography gel depicting μ-calpain and m-calpain activity in sarcoplasmic extracts of the chicken breast muscle incubated at 4 °C in solutions containing 100 mM NaCl and 2 mM NaN_3, with or without caspase 3 inhibitor (DEVD–CHO). Lanes from left to right are: 0 d control (A), 1 d control (B), 1 d DEVD–CHO treatment (C), 3 d control (D), 3 d DEVD–CHO treatment (E), 7 d control (F), and 7 d DEVD–CHO treatment (G). Every lane was loaded with 40 μg of protein.

degradation between treated and control samples was statistically significant ($p < 0.01$).

3.2. Casein zymography

In order to investigate the effects of DEVD–CHO treatment on calpains, a casein zymogram was obtained to visualize activity and molecular changes during *post mortem* aging. After staining, except for at-death samples, both m-calpain and μ-calpain appeared as clear bands, with the former having a higher mobility than the latter (Fig. 6). Also, up to 7 d *post mortem*, there was not a great change in the activity of μ-calpain, whereas the activity of m-calpain increased considerably with extended incubation time, possibly due to m-calpain occurring as a copolymer or zymogen and bearing only limited activity of protein proteolysis in the early stage of chicken *post mortem* storage. In the present study, there was no statistical difference between μ-calpain and m-calpain activities at the same aging time points ($p > 0.05$), indicating that DEVD–CHO has little influence on calpain activity.

4. Discussion

Conversion of muscle to meat is mediated by complex interactions of biochemical processes that take place during *post mortem* storage of the carcass (Herrera-Mendez et al., 2006; Ouali et al., 2006; Takahashi, 1996). Although their influence on the final texture and tenderness of meat is not fully resolved, it is well documented that the proteolysis of some key skeletal myofibril proteins and fragmentation of myofibrils are closely related to the tenderness of aged meat (Huff-Lonergan et al., 1996a; Taylor et al., 1995; Toldr & Flores, 2000). A number of previous studies indicate that proteolytic degradation of structural proteins, including titin, nebulin, troponin-T, desmin, filamin and vinculin, plays a central role in the development of meat tenderness (Koohmaraie, 1996).

Titin is the largest molecule within muscle tissue, with a molecular weight of ca. 3000 kDa. It has been speculated that, in vertebrates, the giant filamentous protein titin (also known as connectin), the only known structure spanning the entire half sarcomere, may act as a molecular blueprint to direct sarcomere assembly (Gregorio, Granzier, Sorimachi, & Labeit, 1999). In this role, titin would coordinate the precise assembly of the structural, regulatory and contractile proteins within the sarcomere, a prerequisite for efficient contraction. Nebulin, an 800 kDa thin-filament-binding protein, specific to skeletal muscle, forms the fourth structural filament system of striated muscle. This protein is suggested to function as a molecular ruler to specify the length of thin-filaments (McElhinny, Kazmierski, Labeit, & Gregorio, 2003). Desmin, with molecular weight of approximately 53 kDa, is a muscle-specific protein and a key subunit of the intermediate filament in cardiac, skeletal and smooth muscles. It plays an essential role in maintaining muscle cytoarchitecture by forming a three-dimensional scaffold around the myofibrillar Z-disk and by connecting the entire contractile apparatus to the subsarcolemmal cytoskeleton, the nuclei, and other organelles (Baron et al., 2004). Troponin-T is the tropomyosin-binding component of the troponin complex, which is involved in the calcium dependent regulation of skeletal muscle contraction. Many isoforms occur for troponin-T, with molecular weights ranging from 45 to 32 kDa (Ho et al., 1994). Because of structural importance, it is conceivable that the degradation of these skeletal proteins will inevitably lead to the disruption and fragmentation of myofibrils within muscle fibre and, as a result, to tender meat. Although it is well documented that proteolysis is a major biological process contributing to meat *post mortem* aging, there is still dispute about which enzymes are involved in the process (Ouali et al., 2006; Sentandreu, Coulis, & Ouali, 2002). More recently, through in *vitro* and in *vivo* experiments, Koohmaraie and others have demonstrated that the muscle endogenous calpains play a central role in meat *post mortem* tenderization, while other endogenous enzymes, such as lysosomal cathepsins and proteosome, contribute little to meat tenderization (Geesink et al., 2001; Koohmaraie, 1996). However, to our knowledge, there is no direct evidence proving that the apoptosis effective proteases, caspases, play a role in muscle *post mortem* proteolysis. In our present study, when a highly specific, potent and reversible inhibitor of caspase3 DEVD–CHO (Nicholson et al., 1995) was incorporated into the incubation solution, the degradations of titin, nebulin, desmin, and troponin-T were significantly inhibited. Although troponin-T is not believed to be an important structure-maintaining protein, the 30 kDa fragment derived from the parent troponin-T was shown to be an indicator of meat aging and tenderization, due to its close relationship to meat tenderness (Huff-Lonergan et al., 1996a). In addition, other reports have documented that executioner (effector) caspases are able to cleave cell skeletal proteins, such as actin, spectrin and desmin, etc. (Chen, Chang, Trivedi, Capetanaki, & Cryns, 2003; Du et al., 2004; Mashima, Naito, Fujita, Noguchi, & Tsuruo, 1995). To further evaluate whether DEVD–CHO influences the activity of calpains, we analyzed the activity of calpains by casein zymography. Results showed that there was no statistical difference between treated and control samples during the whole incubation period. Therefore, the retarded degradation of skeletal proteins cannot be attributed to the calpain activity in treated samples. Consequently, it can be concluded that caspase 3 is possibly involved in myofibrillar protein digestion.

5. Conclusions

In summary, the present results have demonstrated that the selective inhibitor of apoptosis, executioner caspase3, markedly inhibited the degradation of muscle skeletal proteins, titin, nebulin, desmin and troponin-T, which are degraded naturally in *post mortem* skeletal muscles. Moreover, the activity of calpains was not affected by applying DEVD–CHO. Therefore, the degradation of muscle proteins should not be exclusively attributed to the calpain system, and the effector caspase3 may be involved in muscle protein proteolysis. In order to fully elucidate the role of caspase3, further experiments should be performed to characterize the activation and myofibril degradation profiles of caspase3, which is being undertaken in our laboratory.

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