Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 94 (2006) 7–13
Food Chemistry
www.elsevier.com/locate/foodchem

Degradation of myofibrillar proteins by a myofibril-bound serine proteinase in the skeletal muscle of crucian carp (*Carasius auratus*)

Min-Jie Cao [a,*], Xin-Jing Jiang [a,b], Hui-Chang Zhong [a], Zhi-Jun Zhang [c], Wen-Jin Su [a]

[a] *College of Biological Engineering, Jimei University, Jimei, Xiamen, Fujian 361021, China*
[b] *College of Food Science, Shanghai Fisheries University, Shanghai 200090, China*
[c] *School of Life Science, Nanjing University, Nanjing 210093, China*

Received 10 May 2004; received in revised form 10 September 2004; accepted 10 September 2004

Abstract

A myofibril-bound serine proteinase (MBSP) in the skeletal muscle of crucian carp (*Carasius auratus*) was identified. Hydrolysis of myofibrillar proteins by the endogenous MBSP was studied. Myosin heavy chain (MHC) was degraded markedly when crucian carp myofibril was incubated at 55 °C, as shown by SDS-PAGE. Prolonged incubation of myofibril at 55 °C also caused the obvious degradation of tropomyosin, while the decomposition of other myofibrillar proteins, such as α-actinin and actin, was slight, as detected by Western blotting. The results suggest the existence of an endogenous myofibril-associated proteinase in crucian carp myofibril, which efficiently cleaves MHC and tropomyosin. Serine proteinase inhibitors (Lima bean trypsin inhibitor, PMSF and benzamidine) greatly suppressed the degradation of MHC, caused by the enzyme, while inhibitors for cysteine, metallo-, and aspartic proteinases showed only partial or incomplete inhibitory effects, indicating that the endogenous proteinase is a serine proteinase. Substrate specificity analysis, using partially purified crucian carp MBSP, suggested that the enzyme is a trypsin-like serine proteinase.

Keywords: Crucian carp; Degradation; Myofibril; Inhibition; Serine proteinase

1. Introduction

Crucian carp (*Carasius auratus*) is a popular cultured freshwater fish species in different provinces of China. Freshwater fish constitute a potential raw material for surimi production. However, as with marine fish, the modori phenomenon (thermal gel degradation of fish jelly products at around 55 °C) also occurs in the manufacturing process using freshwater fish flesh as the raw material (Cao et al., 1999; Shimizu, Machida, & Takenami, 1981). As the modori effect markedly reduces the commercial value of fish jelly products, the cause of this effect has been studied. The breakdown of myosin heavy chain (MHC) is known to be the main cause of the modori-phenomenon, as MHC is essential for fish gel formation. Researches have indicated that the degradation of MHC is caused by endogenous proteinases, such as cathepsins (An, Weerasinghe, Seymour, & Morrissey, 1994; Nakamura, Ogawa, Saito, & Nakai, 1998) or serine proteinases (Kinoshita, Toyohara, & Shimizu, 1990; Shimizu, Nomura, & Nishioka, 1986; Yanagihara, Nakaoka, Hara, & Ishihara, 1991), especially myofibril-bound serine proteinases (Cao et al., 1999, Cao, Osatomi, Hara, & Ishihara, 2000; Osatomi, Sasai, Cao, Hara, & Ishihara, 1997).

Though a previous study suggested the involvement of cathepsin (cathepsin L) in the modori effect (An et al., 1994), we regard this possibility as quite low,

* Corresponding author. Tel.: +86 592 6180378; fax: +86 592 6180470.
E-mail address: mjcao@jmu.edu.cn (M.-J. Cao).

0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.09.016

because repeated washing using water or low alkaline water is a necessary procedure in the production of fish jelly products. Thus, the existence of soluble lysosomal proteinases (mainly cathepsins) in the final product is actually negligible. Moreover, cathepsin L shows activity only under acidic conditions (Ogata, Aranishi, Hara, Osatomi, & Ishihara, 1998) and will greatly lose its activity in the temperature range 55–60 °C.

Like other intracellular proteins, myofibrillar proteins are also in a dynamic state of degradation and resynthesis. Studies on the metabolism of myofibrillar proteins have indicated that, though lysosomal enzymes play important roles in the turnover of cellular proteins, they only account for a fraction of intracellular protein degradation and that there are other pathways for this degradation, such as the Ca^{2+}-dependent cystein proteinase (calpain) pathway (Tidball & Spencer, 2000), ATP/ubiquitin-dependent proteinase (proteasomes) pathway (Orlowski, 1990; Solomon & Goldberg, 1996) and serine proteinase pathway(s) (Hori et al., 2002). Among serine proteinases investigated, both trypsin-like (Kay, Siemankowski, Siemankowski, & Goll, 1982a, 1982b) and chymotrypsin-like proteinases (Holt et al., 1998; Hori et al., 2002) were identified in the skeletal muscles of mice and hamster, respectively. On the other hand, in the skeletal muscle of fish, the existence of serine proteinases has also been identified (Kinoshita et al., 1990; Shimizu et al., 1986; Toyohara, Sakata, Yamashita, Kinoshita, & Shimizu, 1990; Toyohara & Shimizu, 1988; Yanagihara et al., 1991). More recently, trypsin-like myofibril-bound serine proteinases (MBSP) have been purified to homogeneity, both from freshwater fish common carp (Osatomi et al., 1997) and marine lizard fish (Cao et al., 2000). The proteolytic effect of MBSP on myofibrillar proteins and surimi products analysis revealed that MBSP has the greatest possible influence on fish jelly product elasticity (Cao et al., 1999). In the present study, we identified a proteinase with characteristics similar to MBSP in crucian carp skeletal muscle and studied its involvement in the degradation of myofibrils and individual myofibrillar proteins, such as myosin heavy chain, α-actinin, actin and tropomyosin.

2. Materials and methods

2.1. Materials

2.1.1. Fish

Cultured crucian carp (body weight about 350 g) were obtained alive from a market in Jimei, Xiamen, China. The fish were subdued in iced water and sacrificed instantly. After decapitation and evisceration, the fish were filleted and the fillets were immediately used for myofibril preparation or kept in a −80 °C freezer for further use.

2.1.2. Chemicals

Lima bean trypsin inhibitor (LBTI) was from Worthington biochemical corporation (NJ, USA), phenylmethanesulfonyl fluoride (PMSF) and benzamidine are products from Sigma–Aldrich, L-3-carboxy-*trans*-2, 3-epoxypropionyl-L-leucine-4-guanidinobutylamide (E-64) was a product of Amresco (Solon, OH, USA), pepstatin was purchased from Roche (Mannhem, Germany), EDTA and protein standards for SDS-PAGE were from Bio-Rad (Richmond, CA, USA) and immunoblotting agents were from New England BioLabs (Beverly, MA, USA). Antibodies of rat anti-red sea bream α-actinin and actin were kind gifts from Dr. Tachibana of the Faculty of Fisheries, Nagasaki University, Japan. Antibody of rat anti-carp tropomyosin was prepared in our own laboratory. The second antibody of rabbit anti-rat IgG-HRP was from DAKO (Denmark). Other reagents were all of analytical grade.

2.2. Preparation of myofibrils from skeletal muscle

Crucian carp skeletal muscle (20 g wet weight), stored at −80 °C, was minced and homogenized with four volumes of ice-cold 50 mM phosphate buffer (pH 7.5), using a homogenizer (Kinematica, PT-2100, Switzerland). The homogenization process was performed on ice using a Polytron, PT-DA 2120 at the speed indicator of 15, and the operation was carried out twice (30 s each time and an interval of 1 min). The resulting homogenate was centrifuged at 5000*g*, 4 °C for 15 min in a centrifuge (Avanti J-25, Beckman Coulter, USA). The supernatant was discarded, while the pellet was collected and resuspended in a 4-fold volume of ice-cold phosphate buffer. After three repeated cycles of homogenization and centrifugation, the resulting pellet was suspended in 50 mM phosphate buffer and further homogenized. Finally, after centrifugation at 3000*g* for 15 min, the pellet was resuspended in 50 mM phosphate buffer (pH 8.0) containing 0.5 M NaCl and this suspension was regarded as crucian carp myofibrils. The myofibrils were used immediately for the experiment or stored in a −80 °C freezer for further use. The protein concentration was determined by the method of Lowry, Rosebrough, Farr, and Randall (1951) after 10-fold dilution of the fish myofibril with 50 mM phosphate buffer (pH 8.0) containing 0.5 M NaCl. Bovine serum albumin was used as standard.

2.3. SDS-PAGE analysis and Western blotting

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed under reducing conditions, using 10% mini-slab gels according to the method of Laemmli (1970) and the gels were stained for protein with Coomassie Brilliant Blue R-250. Western blotting was carried out as described by Towbin, Staehelin, and

Gordon (1979). Briefly, myofibrillar proteins on the 10% acrylamide gels were electrophoretically transferred onto a nitrocellulose membrane in transfer solution. Non-specific protein sites were blocked with 5% non-fat milk in Tris–HCl buffered saline (TBS = 20 mM Tris–HCl, pH 7.5, containing 0.145 M NaCl). The blotted proteins were incubated with polyclonal antibodies at room temperature for 2 h and washed with TBST (TBS, 0.05% Tween 20). After incubation for 1 h with horseradish peroxidase conjugated secondary antibody, the nitrocellulose membrane was washed extensively with TBST. Immunodetection was carried out using a detection kit from Bio-Rad.

2.4. Degradation of myofibrillar proteins at different temperatures

To investigate the degradation action of this endogenous proteinase on myofibrillar proteins at different temperatures, 200 μl of crucian carp myofibril (approximately 2 μg protein/μl), dissolved in 50 mM phosphate buffer, pH 8.0, containing 0.5 M NaCl, was incubated in Eppendorf tubes at different temperatures (40, 50, 55, 60 and 70 °C), for 1 h. After incubation, 200 μl of 4× SDS loading buffer was added to each tube and boiled in the presence of 5% 2-mercaptoethanol for 5 min, followed by SDS-PAGE or Western blotting. The degradation of MHC by the endogenous proteinase was observed by SDS-PAGE, while that of other myofibrillar proteins (α-actinin, actin and tropomyosin) was detected by Western blotting, using specific polyclonal antibodies, so that the detection specificity could be enhanced.

2.5. Time-course degradation of myofibrillar proteins

To investigate the degradation of myofibrillar proteins by the endogenous proteinase with time, a time-course study was carried out, using 200 μl myofibril dissolved in 50 mM phosphate buffer, pH 8.0, containing 0.5 M NaCl and incubated at 55 °C for 0, 5, 10, 30 min, 1, 2 and 4 h, respectively. After incubation, 200 μl of 4× SDS loading buffer were added to each tube and boiled in the presence of 5% 2-mercaptoethanol for 5 min, followed by SDS-PAGE and Western blotting.

2.6. Inhibition of myofibrillar protein degradation by different types of inhibitors

To further investigate which type of the endogenous proteinase was responsible for myofibrillar protein degradation, the inhibition effects of different kinds of proteinase inhibitors on the degradation of myosin heavy chain were investigated. The experiment was performed using 100 μl myofibril dissolved in 50 mM phosphate buffer, pH 8.0 containing 0.5 M NaCl and different types of proteinase inhibitors were added. After mixing and incubation at room temperature for 30 min, the mixture was then incubated at 55 °C for 1 h and the inhibition effect on MHC degradation was detected by SDS-PAGE using a 10% mini-slab gel.

2.7. Extraction of myofibril-bound serine proteinase from myofibril

Crucian carp skeletal muscle was homogenized with 4 volumes of 20 mM Tris–HCl buffer, pH 8.0. The homogenate was immediately heated in boiling water while stirring until the temperature reached 55 °C and further incubated at the same temperature for 5 min, followed by immediate cooling in ice water to 4 °C. After centrifugation, the supernatant, which was regarded as crude MBSP, was used for enzymatic characterization.

3. Results and discussion

Myofibrils predominantly consist of myosin heavy chain (MHC, 200 kDa) and actin (42 kDa) and other minor proteins, such as α-actinin (100 kDa) and tropomyosin (34 kDa). In order to elucidate the existence of endogenous proteinase(s) in myofibril and its degradation effect on myofibrillar proteins, crucian carp myofibril (pH 8.0), without the addition of any proteinase inhibitor, was incubated at different temperatures (40, 50, 55, 60 and 70 °C) for 1 h. The results are shown in Fig. 1. Compared with the control for which no incubation was performed, the band of MHC was markedly

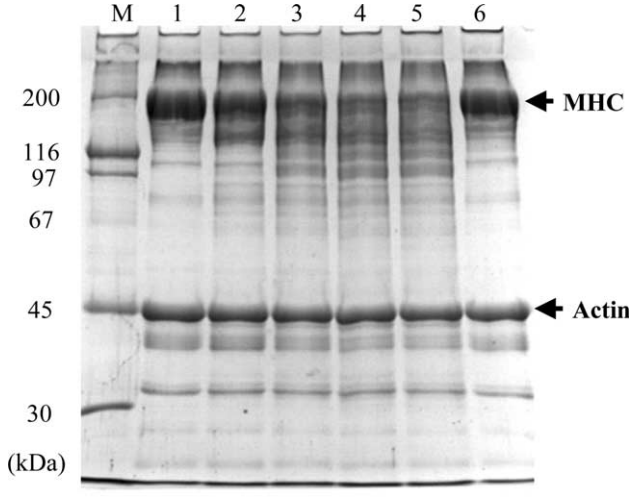

Fig. 1. SDS-polyacrylamide gel electrophoresis of crucian carp myofibrillar proteins. Myofibril dissolved in 50 mM phosphate buffer (pH 8.0) containing 0.5 M NaCl was incubated at 55 °C for 1 h. After incubation, SDS-loading buffer was added to the reaction mixture, followed by boiling for 5 min, samples were applied to a 10% gel. Lanes: (M), molecular weight marker; (1), non-incubated control; (2), 40 °C; (3), 50 °C; (4), 55 °C; (5), 60 °C; (6), 70 °C.

degraded into lower molecular weight products, with sizes ranging from 100 to 200 kDa in the temperature range 50–60 °C. Reduction of MHC at the lower temperature of 40 °C or the higher temperature of 70 °C, however, was not obvious. As the reduction of protein bands in SDS-PAGE is an indicator of proteolytic activity (Yongsawatdigul, Park, Virulhakul, & Viratchakul, 2000), this result suggested that the optimum temperature of the endogenous proteinase involved in the degradation of myofibrillar proteins was around 55 °C. The endogenous proteinase was originally identified in the myofibril fraction, having been homogenized and centrifuged four more times. After this treatment, the remaining sarcoplasmic fraction, if any, is negligible. Thus, it is reasonable to propose that the proteinase(s) responsible for myofibrillar protein degradation is strongly associated with myofibrils.

Degradation of myofibrillar proteins not only happens on MHC (200 kDa), it may also cause the breakdown of other larger molecular mass proteins, such as titin (2000–3500 kDa) and nebulin (650–850 kDa). The degradation products of these higher molecular weight proteins will definitely interfere with our interpretation of the breakdown of other lower molecular weight myofibrillar proteins. Thus, the degradation actions of the endogenous proteinase on α-actinin, actin and tropomyosin were investigated by Western blotting, using specific antibodies against these proteins. It was evident that, after a 1 h incubation of myofibril at 55 °C, the proteins α-actinin, actin and tropomyosin revealed negligible reduction compared to the control (Fig. 2(a)–(c)). These results indicated that the myosin heavy chain is more easily degraded by the endogenous proteinase than the other myofibrillar proteins. As the temperature range of 55–60 °C is a range regarded as a modori-causing range and MHC is the major component in myofibril and provides gel forming ability in the preparation of fish jelly products (Cheng, Hamann, & Webb, 1979; Toyohara & Shimizu, 1988), the MHC breakdown phenomenon at 55–60 °C strongly suggested that the endogenous proteinase is the enzyme responsible for modori.

Further study on the degradation revealed that the reduction of myofibrillar proteins, especially MHC, by the endogenous proteinase is time-dependent. As shown by SDS-PAGE (Fig. 3), the MHC band decreased proportionally with the incubation time at 55 °C and its original band completely disappeared after 4 h. Thus, the endogenous proteinase, not only has high activity around 55 °C, but is also thermally stable at this temperature. Compared with the dramatic degradation of MHC, α-actinin and actin only showed slight decomposition even after prolonged incubation to 4 h (Fig. 4(a) and (b)), while tropomyosin also under dramatic degradation (Fig. 4(c)). These results indicate that MHC and tropomyosin are more easily attacked by the endogenous proteinase than are other myofibrillar proteins. This result strongly suggests that the enzyme is involved in the modori phenomena in fish jelly products production where MHC degradation is the main cause.

In order to know the characteristics of the endogenous proteinase responsible for the degradation of

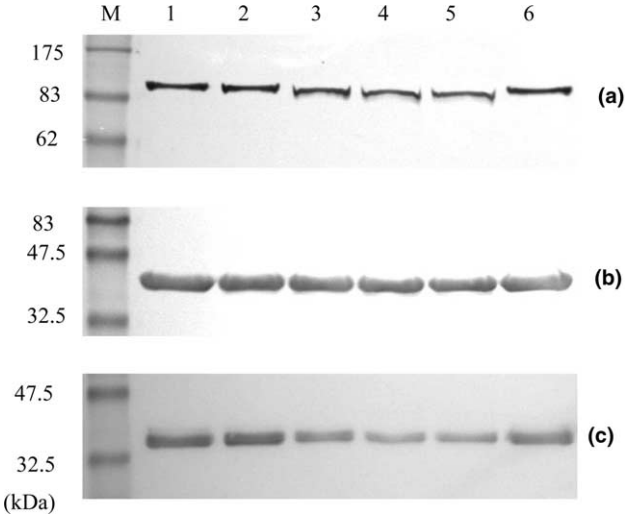

Fig. 2. Western blot of the degradation of α-actinin (a), actin (b) and tropomyosin (c) at different temperatures. Crucian carp myofibril was incubated at different temperatures for 1 h as described in Fig. 1, then applied to SDS-PAGE. After electrophoresis, the gels were transferred to nitrocellulose membranes and reacted with corresponding antibody, followed by immunodetection. Lanes: (M), prestained marker; (1), non-incubated control; (2), 40 °C; (3), 50 °C; (4), 55 °C; (5), 60 °C; (6), 70 °C.

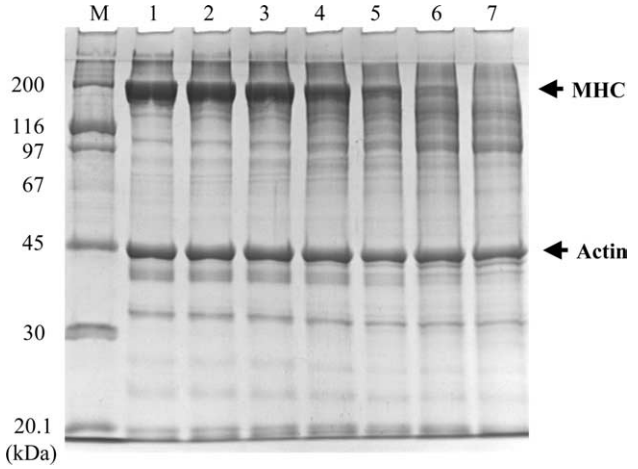

Fig. 3. SDS-polyacrylamide gel electrophoresis of the time-course degradation of crucian carp myofibrillar proteins by the endogenous proteinase. Myofibrils were dissolved in 50 mM phosphate buffer (pH 8.0) containing 0.5 M NaCl and incubated at 55 °C for 0, 5 min, 10 min, 30 min, 1 h, 2 h and 4 h, respectively. After incubation, SDS-loading buffer was added to the reaction mixture, followed by boiling for 5 min, and samples were applied to SDS-PAGE (12% gel). Lanes: (M), molecular marker: (1), 0 h; (2), 5 min; (3), 10 min; (4), 30 min; (5), 1 h; (6), 2 h; (7), 4 h. Arrowheads indicate the positions of myosin heavy chain (MHC) and actin, respectively.

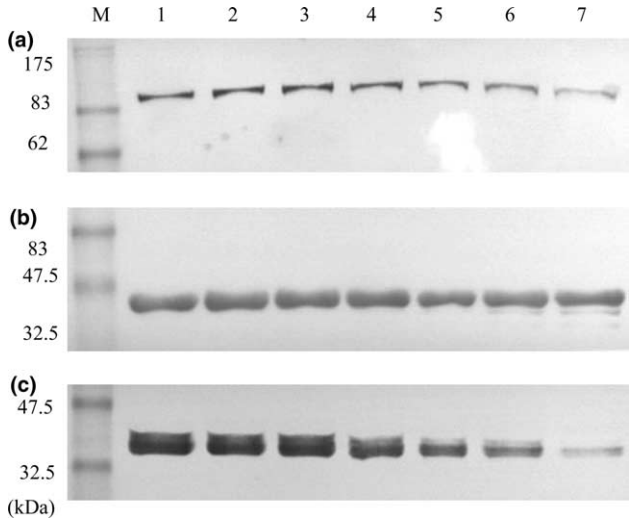

Fig. 4. Western blot of the time-course degradation of α-actinin (a), actin (b) and tropomyosin (c) at 55 °C. Crucian carp myofibril was incubated as described in Fig. 3, then applied to SDS-PAGE. After electrophoresis, the gels were transferred to nitrocellulose membranes and reacted with corresponding antibody, followed by immunodetection. Lanes: M, prestained marker; (1), 0 h; (2), 5 min; (3), 10 min; (4), 30 min; (5), 1 h; (6), 2 h; (7), 4 h.

myofibrillar proteins, different kinds of specific proteinase inhibitors were added to the myofibrils to investigate the suppression effect of these inhibitors on the hydrolytic activity. As shown in Fig. 5, compared to the control (lane 9), to which no proteinase inhibitor was added and which was incubated at 55 °C for 1 h, serine proteinase inhibitors, such as lima bean trypsin inhibitor (LBTI), PMSF and benzamidine suppressed the reduction of myosin heavy chain completely, while pepstatin, an inhibitor for aspartic proteinases and EDTA, an inhibitor for metalloproteinases did not show any inhibitory effect on the suppression of myosin heavy chain degradation. From the above results, it is obvious that the endogenous proteinase responsible for myofibrillar protein decomposition is a serine proteinase. Interestingly, E-64, a specific inhibitor for cysteine proteinases, also showed considerable inhibition. Though the answer to this phenomenon is unknown at present, similar results were also found in the cases of a myofibril-bound serine proteinase from common carp (*Cyprinus carpio*) muscle (Osatomi et al., 1997) and two serine proteinases from rat muscle (Tamanoue, Takahashi, & Takahashi, 1993) where the presence of a critical thiol group(s) near the active site was proposed.

To further characterize the endogenous proteinase, the proteinase was extracted from myofibril by a heating treatment as this process denatured most myofibrillar proteins and the enzyme was partially released. Though the purification of this proteinase to high degree could not be achieved at the present stage, the data on the susceptibility of the crude enzyme to different types of proteinase inhibitors in vitro (Table 1) revealed that the endogenous proteinase is a serine proteinase. This result is in good agreement with its degradation of myofibrillar proteins in the presence of inhibitors. Substrate specificity results (Table 2) showed that the enzyme selectively cleaved at the C terminal of lysine and arginine residues, while no cleavage activity was identified for Suc-Leu-Leu-Val-Tyr-MCA, a substrate of chymotrypsin. Thus, it is evident that the endogenous proteinase is a trypsin-like serine proteinase.

The existence of myofibril-bound serine proteinase (MBSP) has been identified in many kinds of animal. Hori, Ohtani, Hori, and Nokihara (1998) and Holt

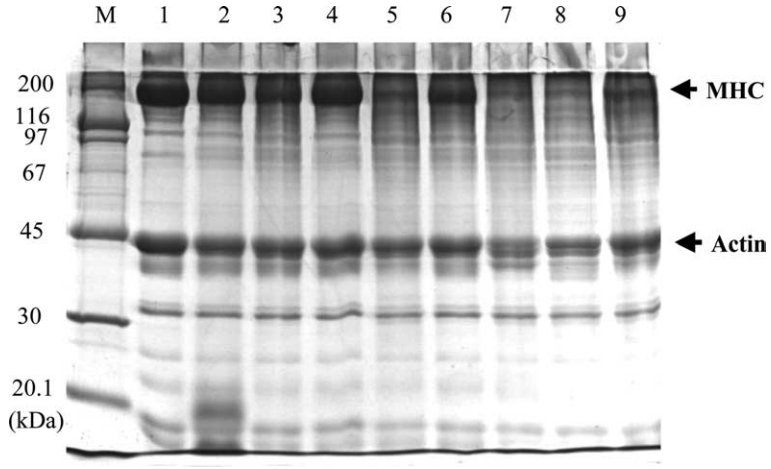

Fig. 5. SDS-PAGE analysis of the inhibitory effect of proteinase inhibitors on the degradation of myofibrillar proteins. Crucian carp myofibrils were dissolved in 50 mM phosphate buffer (pH 8.0) and lima bean trypsin inhibitor (LBTI) to the final concentration of 1 mg/ml; PMSF to 5 mM; Benzamidine to 5 mM; E-64 to 0.1 mg/ml; pepstatin to 10 μg/ml; EDTA to 5 mM, respectively, added to the myofibrils and pre-incubated at room temperature for 30 min followed by incubation at 55 °C for 1 h. Lanes: M, molecular marker; (1) non-incubated control; (2), LBTI; (3), PMSF; (4), benzamidine; (5), E-64; (6), pepstatin; (7), EDTA; (8) control (55 °C, 1 h). Arrowheads indicate the positions of myosin heavy chain (MHC) and actin.

Table 1
Effect of various protein inhibitors on the proteolytic activity of the crude proteinase

Inhibitors	Final concentration (mM)	Relative activity (%)
Control		0
LBTI	0.1 mg/ml	4
PMSF	5.0	12.7
Benzamidine	5.0	20
Chymostatin	0.1	44.7
E-64	0.1 mg/ml	31
Pepstatin	0.1 mg/ml	97.8
EDTA	5.0	95.7

Crude endogenous proteinase was preincubated with each reagent in 20 mM Tris–HCl buffer (pH 8.0) at room temperature for 20 min. Remaining activity was assayed at pH 8.0, 55 °C, using Boc-Phe-Ser-Arg-MCA as substrate. Control tests were performed under identical conditions in the absence of chemicals.

Table 2
Substrate specificity of the endogenous proteinase towards synthetic MCA-substrates

Substrates (final concentration 1 μM)	Relative activity (%)
Boc-Phe-Ser-Arg-MCA	100
Boc-Val-Pro-Arg-MCA	190
Boc-Gly-Arg-Arg-MCA	118
Boc-Val-Leu-Lys-MCA	47.5
Boc-Leu-Lys-Arg-MCA	38.8
Boc-Glu-Lys-Lys-MCA	23.5
Boc-Leu-Arg-Arg-MCA	17.6
Z-Phe-Arg-MCA	18.1
Suc-Leu-Leu-Val-Tyr-MCA	0

Enzymatic activity was determined at pH 8.0, 55 °C.

et al. (1998) purified chymotrypsin-type serine proteinses from the skeletal muscle of mouse and hamster, respectively. Sangorrin, Martone, and Sanchez (2002) partially purified a trypsin-type serine proteinase from the skeletal muscle of mouse. MBSPs were also detected in fish (Ramirrez, Garcia-Carreno, Morales, & Sanchez, 2002; Shimizu et al., 1986; Toyohara et al., 1990; Toyohara & Shimizu, 1988). MBSPs in freshwater fish carp and marine lizard fish have been purified to homogeneity and their characteristics have been studied (Cao et al., 1999; Cao et al., 2000; Osatomi et al., 1997). Both common carp and lizard fish MBSPs are trypsin-type and have optimum temperatures of around 55 °C in the degradation of myofibrillar proteins. These results agree well with our present data. More recently, Ramirrez et al. (2002) reported the reduction of the MHC band in surimi, prepared from Atlantic croaker and Mexican flounder, could be effectively inhibited by the addition of serine proteinase inhibitors from legume seeds. All of these results strongly suggest that endogenous myofibril-bound serine proteinases are ubiquitous in animal muscle.

References

An, H., Weerasinghe, V., Seymour, T. A., & Morrissey, M. T. (1994). Cathepsin degradation of Pacific whiting surimi proteins. *Journal of Food Science, 59*, 1013–1017.

Cao, M. J., Hara, K., Osatomi, K., Tachibana, K., Izumi, T., & Ishihara, T. (1999). Myofibril-bound serine proteinase (MBP) and its degradation of myofibrillar proteins. *Journal of Food Science, 64*, 644–647.

Cao, M. J., Osatomi, K., Hara, K., & Ishihara, T. (2000). Identification of a myofibril-bound serine proteinase (MBSP) in the skeletal muscle of lizard fish Saurida wanieso which specifically cleaves the arginine site. *Comparative Biochemistry and Physiology, 125B*, 255–264.

Cheng, C. S., Hamann, D. D., & Webb, N. B. (1979). Effect of thermal processing on minced fish gel texture. *Journal of Food Science, 44*, 1080–1086.

Holt, J. C., Hatcher, V. B., Caulfield, J. B., Norton, P., Umeda, P. K., Melendez, J. A., et al. (1998). Cloning of the cDNA and nucleotide sequence of a skeletal muscle protease from myopathic hamsters. *Molecular and Cellular Biochemistry, 181*, 125–135.

Hori, S., Ohtani, S., Hori, C., & Nokihara, K. (1998). Purification and characterization of myonase from X-chromosome linked muscular dystrophic mouse skeletal muscle. *Journal of Biochemistry (Tokyo), 123*, 650–658.

Hori, S., Yamada, M., Ohtani, S., Hori, C., Yokomizo, T., Webb, T., & Shimokawa, T. (2002). Myonase is localized in skeletal muscle myofibrils. *Journal of Biochemistry (Tokyo), 132*, 417–425.

Kay, J., Siemankowski, R. F., Siemankowski, L. M., & Goll, D. E. (1982a). Degradation of smooth-muscle myosin by trypsin-like serine proteinases. *Biochemical Journal, 201*, 267–278.

Kay, J., Siemankowski, L. M., Siemankowski, R. F., Greweling, J. A., & Goll, D. E. (1982b). Degradation of myofibrillar proteins by trypsin-like serine proteinases. *Biochemical Journal, 201*, 279–285.

Kinoshita, M., Toyohara, H., & Shimizu, Y. (1990). Purification and properties of a novel latent proteinase showing myosin heavy chain degrading activity from threadfin-bream muscle. *Journal of Biochemistry, 107*, 587–591.

Laemmli, U. (1970). Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. *Nature, 277*(259), 680–685.

Lowry, O. H., Rosebrough, N. J., Farr, A. J., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry, 193*, 265–275.

Nakamura, S., Ogawa, M., Saito, M., & Nakai, S. (1998). Application of polymannosylated cystatin to surimi from roe-herring to prevent gel weakening. *FEBS Letters, 427*, 252–254.

Ogata, H., Aranishi, F., Hara, K., Osatomi, K., & Ishihara, T. (1998). Proteolytic degradation of myofibrillar components by carp cathepsin L. *Journal of the Science of Food and Agriculture, 76*, 499–504.

Orlowski, M. (1990). The multicatalytic proteinase complex, a major extralysosomal proteolytic system. *Biochemistry, 29*, 10289–10297.

Osatomi, K., Sasai, H., Cao, M. J., Hara, K., & Ishihara, T. (1997). Purification and characterization of myofibril-bound serine proteinase from carp Cyprinus carpio ordinary muscle. *Comparative Biochemistry and Physiology, 116B*, 183–190.

Ramirrez, J. A., Garcia-Carreno, E. L., Morales, O. G., & Sanchez, A. (2002). Inhibition of modori-associated proteinases by legume seed extracts in surimi production. *Journal of Food Science, 67*, 578–581.

Sangorrin, M. P., Martone, C. B., & Sanchez, J. J. (2002). Myofibril-bound serine protease and its endogeneous inhibitor in mouse: extraction, partial characterization and effect on myofibrils. *Comparative Biochemistry and Physiology, 131B*, 713–723.

Shimizu, Y., Machida, R., & Takenami, S. (1981). Species variations in the gel-forming characteristics of fish meat paste. *Bulletin of the Japanese Society of Scientific Fisheries, 47*, 95–104.

Shimizu, Y., Nomura, A., & Nishioka, F. (1986). Modori (fish gel degradation occuring at around 60 °C)-inducing property of croaker myosin preparation. *Bulletin of the Japanese Society of Scientific Fisheries, 52*, 2027–2032.

Solomon, V., & Goldberg, A. L. (1996). Importance of the ATP-ubiquitin-proteasome pathway in the degradation of soluble and myofibrillar proteins in rabbit muscle extracts. *Journal of Biological Chemistry, 271*, 26690–26697.

Tamanoue, Y., Takahashi, T., & Takahashi, K. (1993). Purification and characterization of two isoforms of serine proteinase from the microsomal membrane of rat liver. *Journal of Biochemistry, 113*, 229–235.

Tidball, J. G., & Spencer, M. J. (2000). Calpains and muscular dystrophies. *The International Journal of Biochemistry & Cell Biology, 32*, 1–5.

Towbin, H., Staehelin, T., & Gordon, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proceedings of the National Academy of Sciences of the United States of America, 76*, 4350–4354.

Toyohara, H., Sakata, T., Yamashita, K., Kinoshita, M., & Shimizu, Y. (1990). Degradation of oval-filefish meat gel caused by myofibrillar proteinase(s). *Journal of Food Science, 55*, 364–368.

Toyohara, H., & Shimizu, Y. (1988). Relation between the Modori phenomenon and myosin heavy chain breakdown in thread-bream gel. *Agricultural and Biological Chemistry, 52*, 255–257.

Yanagihara, S., Nakaoka, H., Hara, K., & Ishihara, T. (1991). Purification and characterization of serine proteinase from white croaker skeletal muscle. *Nippon Suisan Gakkaishi, 57*, 133–142.

Yongsawatdigul, J., Park, J. W., Virulhakul, P., & Viratchakul, S. (2000). Proteolytic degradation of tropical tilapia surimi. *Journal of Food Science, 65*, 129–133.