Journal of National Fisheries University 68 (1) 1 − 9 (2019)

Identification of Myofibril-bound Serine Protease from Red Seabream (*Pagrus major*) Ordinary Muscle

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Abstract : Myofibrillar proteins were prepared from red seabream (*Pagrus major*) ordinary muscle by washing four times to remove sarcoplasmic proteins. Myosin heavy chain (MHC) of the myofibrillar proteins was degraded at 55℃ without the addition of any protease, and the degree of MHC degradation in red seabream was obviously low in comparison with brushtooth lizardfish (*Saurida* sp.). Specific activity of the red seabream myofibrillar proteins was approximately one-thirtieth that of brushtooth lizardfish. Red seabream myofibril-bound serine protease (MBSP) was solubilized by heat treatment of the washed myofibrillar proteins at 55℃ for 10 min. Hydrolysis of synthetic fluorogenic substrate by both the myofibril-bound and solubilized MBSPs was inhibited by Pefabloc SC, a serine protease inhibitor. Both MBSPs rapidly hydrolyzed synthetic fluorogenic substrates containing arginine residues at the P_1 position. These results indicate that red seabream MBSP is a trypsin-type serine protease, as reported in other fish species, such as white croaker (*Pennahia argentata*) and brushtooth lizardfish, utilized as ingredients of kamaboko.

Key words : red seabream, serine protease, modori, kamaboko

Introduction

Myofibril-bound serine protease (MBSP) was first identified from the myofibrillar fraction of fresh water fish, carp, muscle in 1997[1)]. This protease is tightly bound to myofibrils, and has thus been thought to be a main causative molecule of the modori-phenomenon, characterized by the disintegration of the gel strength of fish meat gel products such as "kamaboko". Subsequently, MBSP was purified from the muscle of brushtooth lizardfish (*Saurida* sp.), which is one of the ingredient marine fish of kamaboko, and the enzymatic characteristics of MBSP were revealed[2)]. Brushtooth lizardfish MBSP specifically hydrolyzed synthetic fluorogenic substrates having arginine residue at the P_1 position, whereas it did not hydrolyze other types of synthetic substrates. The activity of MBSP was inhibited by various serine protease inhibitors such as Pefabloc SC, soybean trypsin inhibitor (STI) and α_1-antitrypsin, but was not significantly suppressed by inhibitors of cysteine protease, aspartic protease and metallo protease. Brushtooth lizardfish MBSP is composed of two 28 kDa subunits (homodimer) containing each enzymatic catalytic site. Similar enzymatic characteristics of MBSP have also been reported for gin-buna (*Carassius auratus langsdorfii*)[3)], goldfish (*Carassius auratus auratus*)[4)], wanieso lizardfish (*Saurida wanieso*)[5)] and white croaker (*Pennahia argentata*)[6)]. In addition, it was reported that three amino acid residues, namely His, Asp and Ser, comprising the catalytic site of trypsin-type serine proteases and their neighboring amino acid sequences are conserved in the primary structure of goldfish MBSP[4)]. Based on these results, MBSP has been suggested to be a novel trypsin-type serine protease[1, 2)].

In serial studies on the modori-phenomenon, it was reported that disintegration of kamaboko gel strength is caused by degradation of myosin heavy chain (MHC) at temperatures of 50 to 60℃ during the heating process of

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kamaboko production[7–9]. Furthermore, it is known that the modori–phenomenon is suppressed by the addition of soybean protein containing STI, suggesting that the modori–phenomenon is caused by endogenous fish muscle serine protease[10]. Our previous studies showed that MBSP could not be removed from myofibrillar proteins by washing procedure (sarashi) during kamaboko production, and most rapidly degraded MHC at around 55℃[2,5,11,12]. Because those enzymatic characteristics are consistent with the modori–phenomenon, MBSP has so far been considered to be a modori–inducing protease. However, the relationship between MBSP and the quality of fisheries–processed foods has been investigated in a limited number of taxonomical groups of fish. In this report, in order to obtain more information regarding marine fish MBSP, MBSP was identified from red seabream (*Pagrus major*) muscle and the enzymatic characteristics of red seabream MBSP were investigated.

Materials and Methods

Fish muscle

Cultured red seabream were harvested and immediately killed by cutting the medulla oblongata. Brushtooth lizardfish were obtained from a fish market in Shimonoseki city. Ordinary muscle of both species was collected and immediately stored at −25℃ until use.

Chemicals

Synthetic fluorogenic substrates and E–64 were purchased from Peptide Institute Inc (Osaka, Japan). Pepstatin A, 4– (2–Aminoethyl) –benzensulfonyl fluoride hydrochloride (Pefabloc SC) and Ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma–Aldrich Co., Tokyo, Japan and Fujifilm Wako Pure Chemical Co., Osaka, Japan, respectively. All other reagents used were of reagent grade.

Preparation of myofibrillar proteins and solubilized MBSP solution

All procedures were carried out at 4℃ except for those described below. Approximately 50 g of frozen red seabream muscle was homogenized in a 4–fold volume of 10 mM $Na_2B_4O_7$–KH_2PO_4 buffer (pH 8.0) and centrifuged at 9,000 x g for 20 min. The precipitate was homogenized with a 4–fold volume of distilled water and centrifuged at 9,000 x g for 20 min, and this washing procedure was repeated three times to obtain washed myofibrillar proteins. To prepare solubilized MBSP, the washed myofibrillar proteins were homogenized in a 4–fold volume of distilled water and adjusted to pH 6.0 with 1 M HCl. Then the homogenate was heated at 55℃ for 10 min in boiling water and immediately cooled on ice. Denatured myofibrillar proteins were discarded by centrifugation at 9,000 x g for 20 min and approximately 150 ml of the supernatant was obtained and lyophilized. The lyophilized powder was dissolved in 5 ml of distilled water and this solution was used in the following experiments as solubilized MBSP.

Degradation of myofibrillar proteins

The washed myofibrillar proteins described above were homogenized in a 4–fold volume of 20 mM KH_2PO_4–Na_2HPO_4 buffer (pH 7.5) containing 0.5 M NaCl, and then diluted with the same buffer to a protein concentration of 2 mg/ml to prepare a solution of myofibrillar proteins. One hundred μl of the solution was incubated at 55℃ for 0 to 6 hours. The reaction was stopped by adding 100 μl of sodium dodecyl sulfate (SDS) reagent composed of 0.125 M Tris–HCl buffer (pH 6.8) containing 60% glycerol, 3% SDS and 0.03% bromophenol blue. Degradation of myofibrillar proteins was assessed using a SDS polyacrylamide gel electrophoresis according to the method of Laemmli[13].

Assay of enzymatic activity

The proteolytic activity of the myofibril–bound and solubilized MBSPs was assayed according to our previous report[2] with slight modifications using Boc–Phe–Ser–Arg–MCA as a substrate. One hundred μl of the solution of myofibrillar proteins or solubilized MBSP was mixed with 100 μl of 50 μM Boc–Phe–Ser–Arg–MCA, 500 μl of 100 mM $Na_2B_4O_7$–KH_2PO_4 buffer (pH 8.0), and 300 μl of distilled water, and then incubated at 55℃ for 60 min.

The reaction was terminated by the addition of 1.5 ml of stop solution, containing 30% methanol and 35% 1-butanol, and the released 7-Amino-4-methylcoumarin, AMC, was measured with a spectrofluorometer RF-5300PC, Shimadzu Co., Kyoto, Japan, at an excitation wavelength of 380 nm and an emission wavelength of 470 nm. One unit of enzyme activity was defined as the amount of enzyme releasing 1 nmol of AMC per minute.

Protein concentration

Protein concentrations were determined according to the method of Lowry et al.[14] using bovine serum albumin as a standard.

Results

Comparison of specific activity of myofibril-bound MBSP

The Boc-Phe-Ser-Arg-MCA hydrolyzing activity of myofibril-bound MBSP in myofibrillar proteins of red seabream and brushtooth lizardfish at 55℃ was compared (Fig. 1). Specific activity of red seabream and brushtooth lizardfish was 0.0015 and 0.0470 units/mg, respectively. The brushtooth lizardfish myofibrillar proteins showed approximately 30-fold greater specific activity than red seabream.

Degradation of myofibrillar proteins by myofibril-bound MBSP

As shown in Fig. 2A, when the homogenate of myofibrillar proteins of red seabream was incubated at 55℃, MHC was gradually degraded during the incubation period, whereas actin was not obviously degraded. In brushtooth lizardfish, considerable degradation was observed for MHC within 1 h, and degradation of MHC and the appearance of MHC fragments proceeded over the next 5 h, whereas no obvious change was detected in actin until 6 h (Fig. 2B).

Substrate specificity

Red seabream myofibril-bound MBSP showed proteolytic activity for a variety of synthetic fluorogenic substrates (Table 1). It showed greatest hydrolyzing activity against Boc-Arg-Val-Arg-Arg-MCA, and the activity was approximately 6- to 10-fold that of the other synthetic fluorogenic substrates. On the other hand, solubilized red seabream MBSP relatively rapidly hydrolyzed substrates having arginine residue at the P_1 position in compared to substrates having lysine residue and hydrophobic residues at the P_1 position. Brushtooth lizardfish myofibril-bound MBSP showed similar substrate specificity to our previous report of purified brushtooth

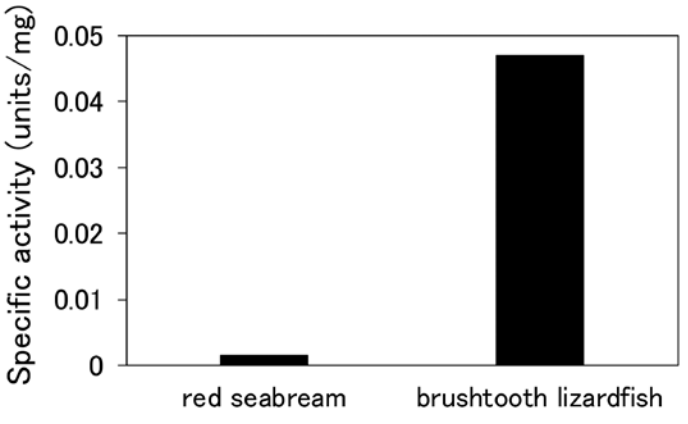

Fig. 1. Specific activity of myofibril-bound MBSPs of red seabream and brushtooth lizardfish myofibrillar proteins for Boc-Phe-Ser-Arg-MCA at 55℃. Specific activity is defined as a units per 1 mg of myofibrillar proteins.

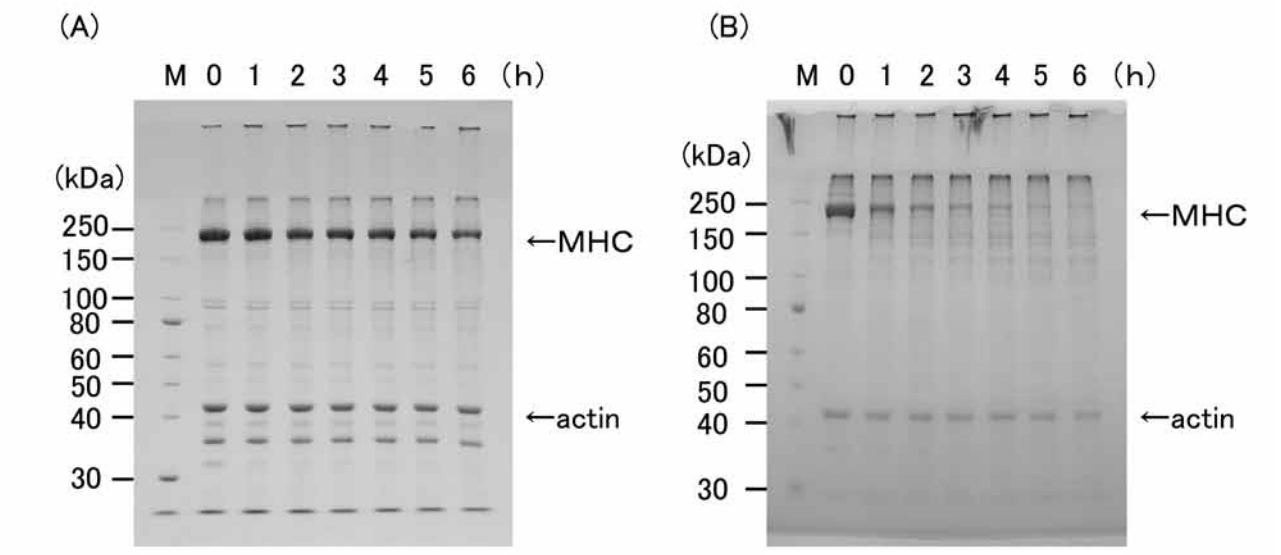

Fig. 2. Degradation of myofibrillar proteins of red seabream (A) and brushtooth lizardfish (B) muscle at 55℃. Approximately 10 μg of myofibrillar proteins was applied to a 7.5–15% gradient polyacrylamide gel. Lane M shows molecular weight markers and the numbers above lane show the incubation periods (hours) at 55℃. Myosin heavy chain (MHC) and actin are shown with allows.

Table 1. Substrate specificity of myofibril-bound and solubilized MBSPs prepared from red seabream and brushtooth lizardfish muscle

Substrate	Relative activity (%)			
	red seabream		brushtooth lizardfish	
	myofibril-bound MBSP	solubilized MBSP	myofibril-bound MBSP	purified enzyme*
Boc-Phe-Ser-Arg-MCA	100	100	100	100
Boc-Val-Pro-Arg-MCA	103	208	200	89
Boc-Gln-Gly-Arg-MCA	86	55	42	106
Boc-Arg-Val-Arg-Arg-MCA	745	84	49	23
Boc-Gly-Arg-Arg-MCA	50	61	32	53
Boc-Gln-Arg-Arg-MCA	165	190	153	106
Boc-Leu-Arg-Arg-MCA	55	101	58	73
Boc-Val-Leu-Lys-MCA	75	33	7	0
Boc-Glu-Lys-Lys-MCA	72	21	4	0
Suc-Leu-Leu-Val-Tyr-MCA	104	8	3	0
Suc-Ala-Ala-Pro-Phe-MCA	66	25	4	0

* Data from Ohkubo et al.[2)]

lizardfish MBSP[2)]. It showed hydrolyzing activity to substrates having arginine residue at the P_1 position, whereas negligible activity was detected for substrates having lysine residue and hydrophobic residues at the P_1 position.

Effects of protease inhibitors

Effects of various protease inhibitors on the activities of myofibril-bound and solubilized MBSPs are shown in Table 2. The activities of both red seabream MBSP were inhibited by Pefabloc SC (a serine protease inhibitor).

Table 2. Effect of various protease inhibitors on the activity of myofibril-bound and solubilized MBSPs prepared from red seabream and brushtooth lizardfish muscle

Inhibitor	Final concentration (mM)	Target protease	Relative activity (%)			
			red seabream		brushtooth lizardfish	
			myofibril-bound MBSP	solubilized MBSP	myofibril-bound MBSP	purified enzyme*
none			100	100	100	100
Pefabloc SC	1	Ser	3	0	4	0
E-64	0.1	Cys	60	89	60	88
Pepstatin A	0.1	Asp	56	104	90	100
EDTA	1	metal	45	44	68	91

Proteolytic activity was measured using Boc-Phe-Ser-Arg-MCA as a substrate, except for red seabream myofibril-bound MBSP, in which Boc-Arg-Val-Arg-Arg-MCA was used.
* Data from Ohkubo et al.[2)]

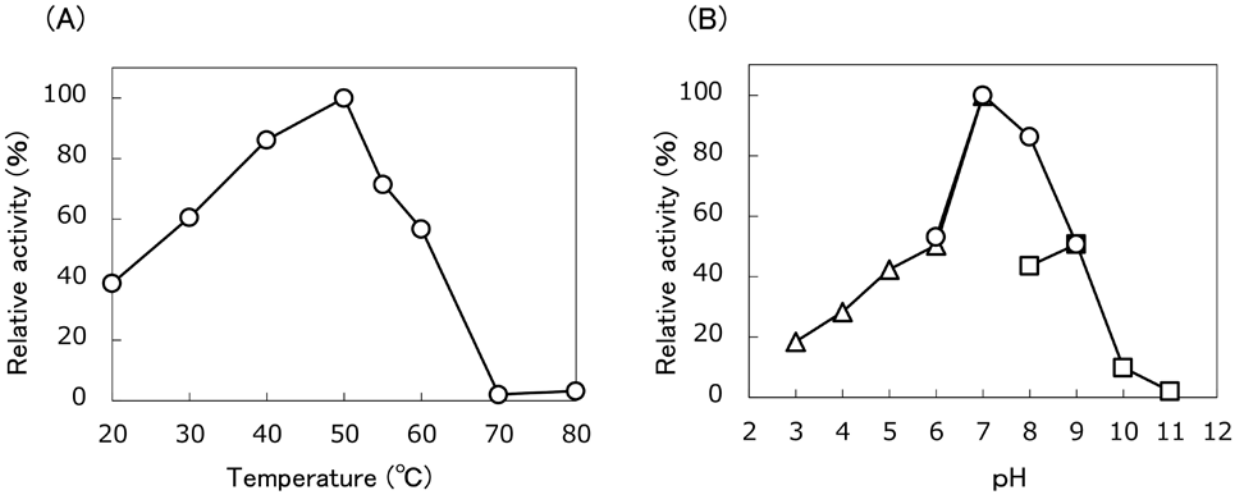

Fig. 3. Effect of temperature (A) and pH (B) on Boc-Phe-Ser-Arg-MCA hydrolyzing activity of solubilized red seabream MBSP. The enzyme activity was measured in 50 mM $C_6H_8O_7$-Na_2HPO_4 buffer (△), $Na_2B_4O_7$-KH_2PO_4 buffer (○), and $Na_2B_4O_7$-KCl-Na_2CO_3 buffer (□).

Almost half of the activity of myofibril-bound MBSP was suppressed by E-64 (a cysteine protease inhibitor), Pepstatin A (an aspartic protease inhibitor), and EDTA (a metallo protease inhibitor). Solubilized MBSP was not inhibited by E-64 and pepstatin A, while approximately half activity was inhibited by EDTA. Both myofibril-bound and solubilized brushtooth lizardfish MBSPs were inhibited by Pefabloc SC, and myofibril-bound MBSP was moderately inhibited by E-64 and EDTA.

Optimum pH and temperature

Optimum pH and temperature of solubilized red seabream MBSP for fluorogenic substrate was investigated (Fig. 3). Solubilized red seabream MBSP showed a highest activity at 50℃. The enzyme showed a highest activity at pH 7.0, and the activity was lower at over pH 9.0 and under pH 6.0. These enzymatic characteristics are similar to those of brushtooth lizardfish MBSP[2)] and white croaker MBSP[6)].

Discussion

MBSP is tightly bound to myofibrillar proteins and is not solubilized even in the presence of detergents[8)]. Thus, it has so far been considered that MBSP is a main

causative molecule of the modori-phenomenon[11,12]. However, reports of the enzymatic characteristics are from limited fish species[1-3,5,6]. In this study, we detected proteolytic activity in red seabream myofibrillar proteins (Fig. 1). Wu et al. reported that a collagenolytic serine protease, hyaluronan binding protein 2 (HABP2), is present in the sarcoplasmic fraction of red seabream muscle, and HABP2 has the ability to degrade MHC[15]. However, HABP2 can be removed by repeated washing of the myofibrillar proteins. Wu et al.[15] also reported that prepared red seabream MHC was degraded at 37℃ without the addition of any protease. But in that report presence of any proteolytic activity in the myofibrillar proteins did not reported. We observed degradation of MHC during incubation of the washed myofibrillar proteins of red seabream at 55℃, which is known as the temperature of modori-phenomenon (Fig. 2A). Furthermore, we detected synthetic fluorogenic substrate hydrolyzing activity in the washed myofibrillar proteins (Fig. 1 and Table 2). These results indicate that MBSP is present in the red seabream myofibrillar proteins. The specific activity of red seabream myofibril-bound MBSP was one-thirtieth that of brushtooth lizardfish (Fig. 1). It is noteworthy that the specific activity of myofibril-bound MBSP are consistent with the degree of MHC degradation at 55℃ between the two species (Fig. 2). It is known that fish meat characteristics including the occurrence of the modori-phenomenon differ among fish species[16]. Therefore, it is suggested that differences in the degree of the modori-phenomenon may be closely related to the activity of MBSP in fish muscle.

Our previous report showed that marine fish MBSP could be solubilized from myofibrils by heat treatment of myofibrillar proteins at 55℃[2,5,6]. Red seabream MBSP was also solubilized by the same procedure, and its enzymatic characteristics were investigated (Tables 1 and 2). The substrate specificity of solubilized red seabream MBSP is similar to that of other fish species such as carp[1], crucian carp[3], white croaker[6] and wanieso lizardfish[5]. The MBSP preferentially hydrolyzed substrates having an arginine residue at the P_1 position (substrates for trypsin-type serine protease) and moderately hydrolyzed substrates with lysine residue (also substrates for trypsin-type serine protease) (Table 1). The activity of the red seabream solubilized MBSP was almost completely inhibited by Pefebloc SC, whereas it was not significantly suppressed by E-64 and Pepstatin A (Table 2). In addition, about a half of the activity was suppressed by EDTA. These results indicate that red seabream MBSP is a trypsin type-serine protease, and that there dose not appear to be significant differences in the enzymatic characteristics of MBSP among fish species. Notably, the substrate specificity and sensitivity to protease inhibitors of the red seabream solubilized MBSP are inconsistent with myofibril-bound MBSP. Our previous studies showed that the activity of brushtooth lizardfish MBSP was affected by the addition of other proteins to the reaction mixture[2]. The substrate specificity of brushtooth lizardfish MBSP is also inconsistent among the myofibril-bound MBSP, crude enzyme (solubilized MBSP), partially purified enzyme, and the purified enzyme (Table 2 and Ohkubo et al.[2]). Hu et al. reported the presence of cathepsin L in the actomyosin fraction in walleye pollock (*Gadus chalcogrammus*) surimi; however, the enzyme was not bound to myofibrils[17]. Liang et al., reported the presence of myofibril-bound EDTA-sensitive protease (MBESP) in the myofibrillar proteins prepared from yellowtail (*Seriola quinqueradiata*) muscle[18]. It is possible that certain proteolytic enzyme may be present in myofibrillar proteins of red seabream in addition to MBSP. Further studies on enzymatic characteristics using purified red seabream MBSP is necessary.

Solubilized red seabream MBSP showed a highest activity against Boc-Phe-Ser-Arg-MCA around 50℃ and over a half of the activity was remand at 60℃, which is known as the temperature of modori-phenomenon (Fig. 3A). The enzyme showed a highest activity at pH 7.0 (Fig. 3B). These results are consistent with results described above that red seabream myofibril-bound MBSP degraded MHC at 55℃ (Fig. 1), and suggested that red seabream MBSP participates in modori-phenomenon. Furthermore, solubilized red seabream MBSP showed a weak activity at 20℃. Our previous

report also demonstrated that brushtooth lizardfish MBSP was gradually degraded MHC at 20℃ over 24 hours[2]. Yoshida et al. reported that a post-mortem auto-degradation of a number of myofibrillar proteins such as MHC, β-connectin, troponin I, tropomyosin in the red seabream during storage at 25℃ was caused by endogenous serine protease[19]. Our results suggested that red seabream MBSP is involved in a post-mortem muscle softening of fresh fish during storage.

Acknowledgement

This work was partly supported by JSPS KAKENHI Grant Number 18K05529.

Reference

1) Osatomi K, Sasai H, Cao MJ, Hara K, Ishihara T: Purification and characterization of myofibril-bound serine proteinase from carp *Cyprinus carpio* ordinary muscle. *Comp Biochem Physiol B Biochem Mol Biol*, **116**, 183-190 (1997)

2) Ohkubo M, Miyagawa K, Osatomi K, Hara K, Nozaki Y, Ishihara T: Purification and characterization of myofibril-bound serine protease from lizard fish (*Saurida undosquamis*) muscle. *Comp Biochem Physiol B Biochem Mol Biol*, **137**, 139-150 (2004)

3) Ohkubo M, Osatomi K, Hara K, Nozaki Y, Aranishi F, Ishihara T: Purification and characterization of myofibril-bound serine protease from crucian carp *Carassius giberio langsdorfi* muscle. *Fish Sci*, **70**, 1183-1185 (2004)

4) Guo C, Cao MJ, Liu GM, Lin XS, Hara K, Su WJ: Purification, characterization, and cDNA cloning of a myofibril-bound serine proteinase from the skeletal muscle of crucian carp (*Carassius auratus*). *J Agric Food Chem*, **55**, 1510-1516 (2007)

5) Cao MJ, Osatomi K, Hara K, Ishihara T: Identification of a myofibril-bound serine proteinase (MBSP) in the skeletal muscle of lizard fish *Saurida wanieso* which specifically cleaves the arginine site. *Comp Biochem Physiol B Biochem Mol Biol*, **125**, 255-264 (2000)

6) Ohkubo M, Osatomi K, Hara K, Ishihara T, Aranishi F: A novel type of myofibril-bound serine protease from white croaker (*Argyrosomus argentatus*). *Comp Biochem Physiol B Biochem Mol Biol*, **141**, 231-236 (2005)

7) Shimizu Y, Nomura M, Nishioka F: Modori-inducing property of croaker myosin preparation. *Nippon Suisan Gakkaishi*, **52**, 2027-2032 (1986)

8) Kinoshita M, Toyohara H, Shimizu Y: Diverse distribution four distinct type modori (gel degradation)-inducing proteinases among fish species. *Nippon Suisan Gakkaishi*, **56**, 1485-1492 (1990)

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

10) Toyohara H, Kinoshita M, Shimizu Y, Sakaguchi M: A group of novel latent serine proteinases degrading myosin heavy chain in fish muscle. *Biomed Biochim Acta*, **50**, 717-720 (1991)

11) Cao MJ, Hara K, Osatomi K, Tachibana K, Izumi T, Ishihara T: Myofibril-bound serine proteinase (MBP) and its degradation of myofibrillar proteins. *J Food Sci*, **64**, 644-647 (1999)

12) Ohkubo M, Osatomi K, Hara K, Ishihara T, Aranishi F: Myofibrillar proteolysis by myofibril-bound serine protease from white croaker *Argyrosomus argentatus*. *Fish Sci*, **71**, 1143-1148 (2005)

13) Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680-685 (1970)

14) Lowry OH, Rosebrough NJ, Farr AJ, Randall RJ: Protein measurement with the folin phenol reagent. *J Biol Chem*, **193**, 265-275 (1951)

15) Wu GP, Chen SH, Liu GM, Yoshida A, Zhang LJ, Su WJ, Cao MJ: Purification and characterization of a collagenolytic serine proteinase from the skeletal muscle of red sea bream (*Pagrus major*). *Comp Biochem Physiol B Biochem Mol Biol*, **155**, 281-287 (2010)

16) Shimizu Y, Nishioka F, Machida R, Shiue CM: Gelation characteristics of salt-added myosin sol. *Bull Japan Soc Sci Fish*, **49**, 1239-1243 (1983)
17) Hu Y, Morioka K, Ito Y: Actomyosin nonbinding cathepsin L in walleye pollock surimi. *J Food Biochem*, **32**, 143-152 (2008)
18) Liang X, Yoshida A, Osatomi K, Wang Y, Cao MJ, Hara K: Degradation of myofibrils in cultured yellowtail *Seriola quinqueradiata* burnt meat: effects of a myofibril-bound EDTA-sensitive protease. *Fish Sci*, **78**, 147-153 (2012)
19) Yoshida A, Kurihara M, Ogata H, Cao MJ, Osatomi K, Hara K: Proteolytic degradation of myofibrillar components in red sea bream muscle. *Jpn J Food Chem Safety*, **21**, 107-114 (2014)

マダイ筋原線維結合型セリンプロテアーゼの同定

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和文要旨

マダイの筋肉を4回洗浄し，水溶性タンパク質を除去した筋原線維タンパク質を調製した。この筋原線維タンパク質を55℃で加熱した結果，ミオシン重鎖の分解が観察されたことから，マダイ筋肉にも筋原線維結合型セリンプロテアーゼ（myofibril-bound serine protease, MBSP）が存在することが示唆された。マダイの筋原線維のBoc-Phe-Ser-Arg-MCAに対する分解活性はマエソの約1/30であり，SDS-PAGEで観察されたマダイのミオシン重鎖の分解も，マエソと比べて緩やかであった。筋原線維タンパク質を55℃で10分間加熱し，遠心分離で上清を得た。この上清を凍結乾燥し，蒸留水に溶解してMBSPの粗酵素液とした。マダイの筋原線維と結合したMBSP及びMBSPの粗酵素液のプロテアーゼ活性は，セリンプロテアーゼ阻害剤であるPefabloc SCにより阻害され，両酵素はアルギニン残基をP_1部位に持つ蛍光合成基質をよく分解した。MBSPの粗酵素液は，Boc-Phe-Ser-Arg-MCAに対して55℃、pH 7.0で最も高い活性を示した。以上の結果から，マダイのMBSPは，シログチ及びマエソのMBSPと同様にトリプシン型のセリンプロテアーゼであることが示唆された。