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Molecular cloning of a myofibril-bound serine proteinase (MBSP) from common carp (*Cyprinus carpio*) muscle

GUO Chuan, LIANG Yin-long, LIU Guang-ming, SU Wen-jin, CAO Min-jie

(*Key Laboratory of Science and Technology for Aquaculture and Food Safety*, *Fujian Province University*, *School of Biotechnology*, *Jimei University*, *Xiamen* 361021, *China*)

Abstract: Myofibril-bound serine proteinase (MBSP) is a recently identified serine proteinase, which is responsible for myofibrillar protein degradation and gel softening in the preparation of fish cakes. However, the full-length sequence of MBSP has never been reported. In the present study, degenerate primers were designed according to the N-terminal amino acid sequence of carp MBSP and well-conserved active site motif of serine proteinases. Based on RT-PCR and PCR amplification of the cDNA fragment from N-terminal to active site motif and rapid amplification of cDNA end (RACE) of the 3′- and 5′- regions, the full-length cDNA of MBSP was confirmed. Analysis of the nucleotide sequence of carp MBSP revealed that the cDNA clone has an open reading frame of 732 bp encoding a protein of 243 amino acid residues and a signal peptide of 21 amino acid residues. Three residues (His61, Asp107 and Ser197) forming the typical catalytic triad of serine proteinases for functional activity were conserved in the polypeptide sequence. Mature MBSP contains 222 amino acid residues with an estimated molecular weight of about 24.5 ku, which is smaller than that of its native protein (30 ku). The estimated pI of mature MBSP is 10.43. Sequence alignment showed that carp MBSP has identity of 80.6% to crucian carp MBSP, 55.8% to porcine trypsin, 55.3% to bovine trypsin, 53.9% to flounder trypsin and 39.2% to a chymotrypsin type serine proteinase mekratin, which is from the skeletal muscle of hamster. The high content (11.93%) of Lys residue distinguished carp MBSP from other serine proteinases and this may account for its myofibril-binding characteristic.

Key words: *Cyprinus carpio*; cloning; MBSP; homology
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1 Introduction

Many biological processes which require specific and limited proteolysis are mediated by the serine protease family produced in different tissues and cell types[1]. Serine proteases are a group of enzymes that hydrolyze peptide bonds in proteins and participate in a broad range of physiological processes. They are characterized by their similar proteolytic cleavage specificities and the presence of a serine residue in the active site. Processing serine proteinases are represented by yeast Kex2, a Ca^{2+}-dependent subtilisin-like serine protease[2,3] and its mammalian counterpart, which includes furin, PC1 and PC3[4],

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Brief introduction of the author: GUO Chuan (1981 -), male, born in Shantou City, Guangdong Province, Master, interested in biotechnology research

Corresponding author: CAO Min-jie, Tel: 0592 - 6180378, E-mail: mjcao@jmu.edu.cn

as well as later found member of the family LPC/PC7/PC8/SPC7[5], etc. They were extensively studied both enzymatically and genetically[5]. The tissue-specific distributions of some of these enzymes were also investigated by Northern blot and *in situ* hybridization[4]. Investigation of cleavage specificity of these enzymes indicated that the Kex2 family serine proteases correctly processed protein precursors into their mature forms by cleaving mainly at the carboxyl side of dibasic pairs on Lys-Arg and Arg-Arg, as well as between the dibasic pairs or the monobasic sites. Processing serine proteinases had also been partially or completely purified from bovine adrenal chromaffin granule membranes[6], rat liver[7], porcine intestinal mucosa[8], and mouse[9]. Because the region around the catalytic site is known to have well-conserved structural homologies[10], serine proteinases are supposed to have evolved from a same ancestral gene.

Compared with research progress of serine proteases from mammals and yeast cells, only few studies have been performed on serine proteinases from fish[11-13]. In a previous paper, a novel myofibril-bound serine proteinase (MBSP) from common carp (*Cyprinus carpio*) muscle was purified to homogeneity[14]. MBSP was assumed to be responsible for the modori phenomenon (thermal gel degradation of fish jelly products) for it optimally hydrolyzed myosin heavy chain and other myofibrillar proteins such as α-actinin, actin and tropomyosin at 55 ℃[13-15]. The cleavage specificity of carp MBSP toward both dibasic and monobasic fluorescent MCA-substrates and peptides were also investigated and its N-terminal amino acid sequence (40 amino acid residues) was determined[16]. As a myofibril binding type serine proteinase, MBSP was also supposed to be a processing enzyme physiologically. Though MBSP is a unique serine proteinase both in its cleavage specificity and binding characteristic, till now, very few information concerning its full-length primary sequence are available. In order to elucidate the binding characteristic and enzymatic properties of MBSP, it is necessary to obtain its full-length primary amino acid sequence for comparative study. In the present study, the cDNA sequence and deduced amino acid sequence of carp MBSP were determined. Our findings may be helpful in revealing more properties of such myofibril-bound serine proteinase(s).

2 Materials and methods

2.1 Fish

Cultured common carp with body weight of about 500 g was obtained alive from the market of Jimei, Xiamen. The fish was sacrificed and skeletal muscle was immediately collected and frozen in liquid nitrogen for total RNA extraction.

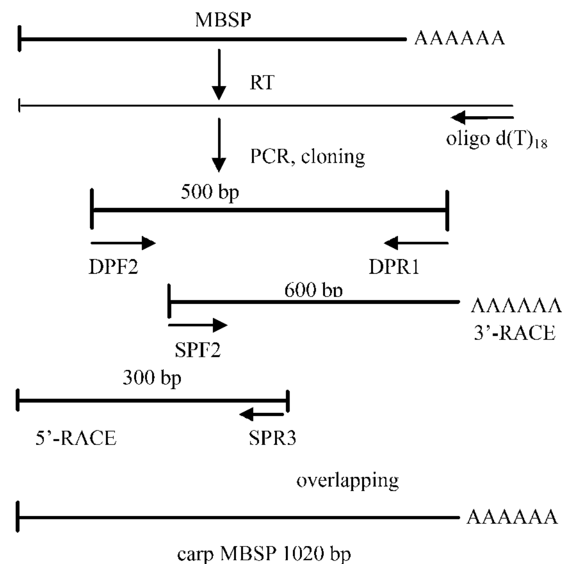

Fig.1 Strategy and relative positions of forward and reverse primers in molecular cloning of carp MBSP
Primers DPF and DPR are degenerate primers SPF and SPR are gene-specific primers

2.2 Total RNA extraction and cDNA cloning

Total RNA was extracted from skeletal muscle with TRIzol reagent (Invitrogen). Approximately 1 μg of RNA was obtained from 1 g of muscle. Using total RNA, first-strand cDNA was synthesized by reverse transcriptase (Invitrogen) to transcribe poly (A) RNA with oligo-d$(T)_{18}$ as primer. Based on the N-terminal amino acid sequence of the purified enzyme[16], two degenerate primers (DPF1, DPF2) were designed for forward primer. From the well-conserved peptide motif GDSGGP in serine protease

family, a degenerate reverse primer (DPR1) was also designed. The strategy for molecular cloning carp MBSP was summarized in Fig.1 while primers used in this study were shown in Tab. 1. The DNA encoding fragment of MBSP was first amplified by PCR using primers DPF1 and DPR1 followed by nested-PCR with primers DPF2 and DPR1. PCR was carried out in 20 μL of reaction mixture composed of 1 μL cDNA or DNA as template, 10 $\mu mol \cdot L^{-1}$ of each primer, 0.25 $\mu mol \cdot L^{-1}$ dNTPs, 1.5 U *Taq* DNA polymerase (Promega) and 2 μL 10 × PCR buffer containing 2.5 $mmol \cdot L^{-1}$ $MgCl_2$ under the following conditions: 35 cycles of denaturation at 94 ℃ for 45 s, 48 ℃ for 45 s and 72 ℃ for 2 min, followed by an extension reaction at 72 ℃ for 7 min. The PCR product was purified from agarose gel and cloned into a pUcm-T easy vector (Sango, Shanghai) and the DNA sequence was determined by ABI 377 DNA sequencer using M13 forward and reverse primers.

2.3 5′-RACE and 3′-RACE

Based on the sequence of cDNA fragment obtained using degenerate primers, specific primers SPF1 and SPF2 (Tab.1) were designed and used as sense primers. 3′-RACE was performed according to the manufacturer's instructions (Clontech, USA) with some modification. Briefly, reverse transcription was carried out using 3′ RACE CDS Primer A to synthesize cDNA. In the first round PCR reaction, SPF1 and NUP Primer (supplied by Clontech) were used. Nested-PCR was carried out using SPF2 and NUP Primers. PCR was carried out in 20 μL of reaction mixture composed of 1 μL cDNA or DNA, 10 $\mu mol \cdot L^{-1}$ of each primer, 0.25 $\mu mol \cdot L^{-1}$ dNTPs, 1.5 U *Taq* DNA polymerase and 2 μL 10 × PCR buffer containing 2.5 $mmol \cdot L^{-1}$ $MgCl_2$ under the following conditions: 35 cycles of denaturation at 94 ℃ for 45 s, 55 ℃ for 45 s and 72 ℃ for 2 min, followed by an extension reaction at 72 ℃ for 7 min. The PCR product was cloned into pUcm-T easy vector and the sequence was determined as described above.

According to the sequence of cDNA fragment, three additional specific antisense primers SPR1, SPR2 and SPR3 were designed (Tab.1) and used in 5′RACE, which was carried out according to the manufacturer's instructions of 5′RACE kit (Clontech, USA) with some modifications. The primer SPR1 was used as gene specific primer in reverse transcription for cDNA synthesis. First PCR amplification and Nested – PCR were performed using SPR2 and SPR3 as reverse primers together with sense primers of UPM and NUP provided in the 5′RACE kit. PCR was carried out in 20 μL of reaction mixture composed of 2 μL cDNA or DNA as template, 10 $\mu mol \cdot L^{-1}$ of each primer, 0.25 $\mu mol \cdot L^{-1}$ dNTPs, 1.5 U *Taq* DNA polymerase and 2 μL 10 × PCR buffer containing 2.5 $mmol \cdot L^{-1}$ $MgCl_2$ under the following conditions: 30 cycles of denaturation at 94 ℃ for 45 s, 54 ℃ for 45 s and 72 ℃ for 2 min, followed by an extension reaction at 72 ℃ for 7 min.

The PCR product from 5′ RACE was also subjected to DNA sequencing as described above.

Tab.1 Primers used in cloning of carp MBSP gene

primers	primer sequences
DPF1	5′-CC(A/T)CA(C/T)AG(C/T)CA(A/G)CC(A/T)TGGCA-3′
DPF2	5′-CT(A/T/C/G)AT(A/T/C)AA(C/T)AA(C/T)CG(A/T/C/G)TGGGT-3′
DPR1	5′-AC(A/T/C/G)GG(C/T)TC(C/T)TC(A/T/C/G)GA(A/G)TC(C/T)TC-3′
3′RACE CDS Primer A	5′-AAGCAGTGGTATCAACGCAGAGTAC(T)30N-1N-3′
SPF1	5′-TGAAGCCAATCCGTCTGCC-3′
SPF2	5′-AGTGCTTGGTTTCTGGATGG-3′
SPR1	5′-CACTACAGGGCCACCTGAAT-3
SPR2	5′-GTTTTTTCAGCTTGATCAGCATGAT-3
SPR3	5′-CTTTGATCTTCTGCTCTG-3′
UPM	5′-CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT-3′ mixed with 5′-CTAATACGACTCACTATAGGGC-3′
NUP	5′-AAGCAGTGGTATCAACGCAGAGT-3′

3 Results

Using RT-PCR and degenerate primers designed on the basis of the N-terminal amino acid sequence of purified carp MBSP and the serine protease active site motif-GDSGGP, we first cloned a DNA fragment of MBSP gene of about 500 bp in length. DNA sequencing analysis of the resulting DNA fragment revealed higher similarity to serine proteinases from other species in GenBank database. This fragment sequence set a solid base for the designation of gene-specific primers, which were subsequently used in 5′ and 3′ RACE.

By nested-PCR in 3′RACE, a DNA fragment of about 600 bp was obtained (Fig.2-A) while the product of nested-PCR in 5′-RACE was 300 bp in length (Fig.2-B). The full-length cDNA sequence of carp MBSP was consequently determined by overlapping the three cDNA fragments.

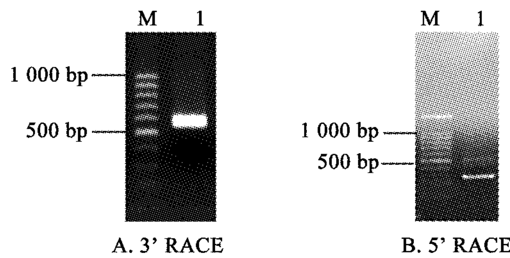

Fig.2 Result of nested-PCR
M: DNA ladder; 1: PCR product of 3′RACE;
2: PCR product of 5′RACE

The complete cDNA sequence and the deduced amino acid sequence of carp MBSP were shown in Fig.3. The full-length cDNA of MBSP was 1020 bp including the start codon ATG at positions of 34 – 36, the stop codon TAA at positions 763 – 765 and a poly A tail. The open reading frame of carp MBSP is 732 bp and the predicted protein consists of 243 amino acid residues (Fig.3). Combining the present DNA sequence with the information of the N-terminal amino acid sequence of native carp MBSP[16], it is obvious that carp MBSP was processed to the mature form of 222 amino acid residues by cleaving a signal peptide of 21 amino acid residues at Lys21 – Ile22. The deduced pI of mature MBSP is 10.43, which is quite alkaline. The molecular weight of MBSP is 24.5 ku as calculated, which is relatively smaller than that of its native form (30 ku)[14]. However, except cysteine residue, the N-terminal sequence (40 amino acid residues) of the deduced mature MBSP revealed 95% identity with Japanese common carp MBSP as determined by protein sequencing[16], further confirming our present data is correct. A homology search in GenBank databases revealed that mature common carp MBSP shares relatively high homology to other serine proteinases especially in the conserved regions (Fig.4). It has 80.6% identity to crucian carp MBSP (GenBank accession No. DQ872434); 55.8% to porcine trypsin[17]; 55.3% to bovine trypsin[18]; 53.9% to flounder trypsin[19] and 39.2% to mekratin, a chymotrypsin-type serine proteinase from the skeletal muscle of hamster[20]. By analyzing the sequence, the characteristic of MBSP as a serine proteinase is very clear. It comprises an activated serine (Ser-197) assisted by a proton relay (His-61), which acts as a general base. The imidazole of the active-site histidine is stabilized by hydrogen bonds to an aspartic acid (Asp-107) (Fig.3). These three residues, brought together by protein folding in a motif known as catalytic triad are well conserved in common carp MBSP.

4 Discussion

Serine proteinases are a group of enzymes that hydrolyze peptide bonds specifically at the carboxyl side of arginine and lysine residues and participate in a broad range of physiological processes. They are present in all forms of living organisms and have been extensively studied because of their biological importance. Though the myofibril-bound serine proteinse of common carp has been purified and its N-terminal amino acid sequence was determined in previous works[14,16], no information concerning the full-length sequence of the proteinase was available. Searching databases in NCBI, SwissProt and DDBJ,

except crucian carp MBSP, no gene with high homology to common carp MBSP was identified. Thus, we believe MBSP is a new member of the serine proteinase family. Interestingly, mature MBSP molecule contains 29 Lys residues in the 222 residues of the mature protein, which accounts for 11.93% in molecular ratio of the total protein. Such a high content of Lys residues is quite rare among known proteins. As carp MBSP is a myofibril-bound serine proteinase and Lys is a hydrophilic amino acid, the high content of Lys may suggest that the binding mode of carp MBSP to myofibril is an ionic interaction. This speculation seems in agreement with the disassociation method of carp MBSP from myofibril during purification in the presence of 0.6 $mol \cdot L^{-1}$ KCl, pH 4.0[14]. However, the possibility of other binding mechanism should not be excluded, because mature carp MBSP molecule as a whole is neutral hydrophilic and contains several hydrophobic domains.

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1      CAACAGATGTGCTCTCTCCTCTCCTCAGTTGCAATGAAGACCACTGTGTTCATCATTCTA
1                                        M  K  T  T  F  I  I  L

61     CTGGTTGCGGTTGTGGCTTTCAGCTCAGGAGATAAAATCATTGGAGGTTATGAATGTAAG
10      L  V  A  V  V  A  F  D  D  G  D  K |I  I  G  G  Y  E  C  K
                         preproregion <----|----> mature form

121    CCCCACTCCCAGCCCTGGCAAGCTTACCTTGTCGATAATAAATTTTCATGTGGAGGATCT
30      P  H  S  Q  P  W  Q  A  Y  L  V  D  N  K  F  S  C  G  G  S

181    TTGATTAATAATAGATGGGTTGTGTCTGCTGCTCAATGCACATTCTCACGTAATAAACTC
50      L  I  N  N  R  W  V  V  S  A  A  H  C  T  F  S  R  N  K  L
                                         ▲
241    AGCGTCCACCTGGGAAGGCACAATTTGCAAACTAATGAAAACACAGGGCAGAAGATCAAA
70      S  V  H  L  G  R  H  N  L  Q  T  N  E  N  T  G  Q  K  I  K

301    GTGGAGAAGATCATTCCTTTCCCGAAATACAATGATAGGCCTCATAACAATGATATCATG
90      V  E  K  I  I  P  F  P  K  Y  N  D  R  P  H  N  N  D  I  M
                                                           ▲
361    CTGATCAAGCTGAAAAAACCTGTCACCTTCAACAAGTATGTGAAGCCAATCCGTCTCATG
110     L  I  K  L  K  K  P  V  T  F  N  K  Y  V  K  P  I  R  L  P

421    AAAAAATGCCCCTCTGTAGGGGAGAAGTGCTTGGTTTCTGGATGGGGCAGAACTGCAGCC
130     K  K  C  P  S  V  G  E  K  C  L  V  S  G  W  G  R  T  A  A

491    GGCTCTGCTTCTGTCCTGCAGTGTTTGAATTTGCCTGTACTCTCACAAAGGACGTGTAAG
150     G  S  A  S  V  L  Q  C  L  N  L  P  V  L  S  Q  R  T  C  K

541    CGTGCGTATAAAAAAATAATAACTAAAAACATGTTCTGCGCTGGATTCATTAAGGGAGGG
170     R  A  Y  K  K  I  I  T  K  N  M  F  C  A  G  F  I  K  G  G

601    AAAGACTCATGCCAGGGGGATTCAGGTGGCCCTCTAGTGTGCGGAGGGCAACTGAAAGGT
190     K  D  S  C  Q  G  D  S  G  G  P  V  V  C  G  G  Q  L  K  G
                             ▲
661    GTTGTTTCCTTTGGCAATGGCTGTGCTAAACCAAAATATCCTGGGGTTTATACTGAGGTG
210     V  V  S  F  G  N  G  C  A  K  P  K  Y  P  G  V  Y  T  E  V

721    TGCCGCTACACTAAATGGATCAAATCCACCATAGCTAAAAACTAACTGTTTATTCAATCT
230     C  R  Y  T  K  W  I  K  S  T  I  A  K  N  *

781    ATTTGAATGAGGAAACATCAAATTCTATAGCAACCAAAGCTTCTAACAGCAGCTGCTGTG
261

841    ATGCAATGACTTGAACAATTAGTGATTGTCTCTTTTATTTAGAAAGCAGGACTATTCATT
291

901    TTCCTTTTTCTATTTTCTTTTGCATTTTTGGGTGCACCATCTTTGTATATCTTAATCTTT
301

961    GAATTAAATTCACTCTGAAATAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
321
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Fig.3　Full length cDNA and deduced amino acid sequence of carp MBSP

▲: catalytic triad

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1  IIGGYECKPHSQPWQAYLVDN.....KFSCGGSLINNRWVVSAAHCTFSRNKLSVHLGRH     55
2  IIGGYECRPHSQPWQAFLTDN.....RISCGGSLINERWAVSAAHCNFQQDRLSVRLGRH     55
3  IVGGYTCAANSIPYQVSLNSG.....SHFCGGSLINSQWVVSAAHC..YKSRIQVRLGEH     53
4  IVGGYTCGANTVPYQVSLNSG.....YHFCGGSLINSQWVVSAAHC..YKSGIQVRLGED     53
5  IIGGVESKPHSRPYMAHLEIVTERGFTASCGGFLITPEFVMTAAHC..KGKEITVTLGAH     58
6  IVGGYECTPHSQAHQVSLNSG.....YHFCGGSLVNENWVVSAAHC..YKSRVEVRMGEH     53

1  NLQTNENTGQKIKVEKIIPFPKYNDRPHNNDIMLIKLKKPVTFNKYVKPIRLP..KKCPS    113
2  NLVTAENTEQRIEAEKMIPFPKYNDRPHNNDIMLIKLKQPATLNRYVKPIPLP..NKCPS    113
3  NIDVLEGNEQFINAAKIITHPNFNGNTLDNDIMLIKLSSPATLNSRVATVSLP..RSCAA    111
4  NINVVEGNEQFISASKSIVHPSYNSNTLNNDIMLIKLKSAASLNSRVASISLP..TSCAS    111
5  DVSKAESTQCKIKVKKQIAHPNYSFYSKLHDIMFLKLQKKAKEDPSVDTIPLPSPSDFLK    118
6  KIRVNEGTEQFVSSSRVIRHPNYDSWNIDNDIMLIKLSKPATLNQYVKTVALP..SSCAP    111

1  VGEKCLVSGWGRTAAG...SASVLQCLNLPVLSQRTCKRAYKKIITKNMFCAGFIKGGKD    170
2  AGEKCLVSGWGRTADG...IASTLQCLKLPVLSEKVCKTAYGSIITRNMFCAGFIRGGKD    170
3  AGTECLISGWGNTKSSGSSYPSLLQCLKAPVLSDSSCKSSYPGQITGNMICVGFLEGGKD    171
4  AGTQCLISGWGNTKSSGTSYPDVLKCLKAPILSDSSCKSAYPGQITSNMFCAGYLEGGKD    171
5  PGKMCRAAGWGRTGVT.EPTSERLREVKLRIMDKGACKNYYH.YHYDFQVCVGSPRRVRS    176
6  AGTMCKVSGWGNTMSS.ADNGDLLQCLDIPILSFSDCNNAYPGMITDSMFCAGYLEGGKD    170

1  SCQGDSGGPVVCGGQLKGVVSFGNGCAKPKYPGVYTEVCRYTKWIKSTIAKN            222
2  SCQGDSGGPVVCKGQLKGVVSFGNGCAKPKYPGVYAEVCRYTRWIKSTIASN            222
3  SCQGDSGGPVVCNGQLQGIVSWGYGCAQKNKPGVYTKVCNYVNWIQQTIAAN            223
4  SCQGDSGGPVVCSGKLQGIVSWGSGCAQKNKPGVYTKVCNYVSWIKQTIASN            223
5  AYKGDSGGPLLCAGVAHGIVSYGRGDAKP..PAVFTRISSYVPWINIVIKAS            226
6  SCQGDSGGPVICNGELQGVVSWGYGCAERGNPGVYAKVCLFNDWLESTMASY            222
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Fig.4 Comparison of the amino acid sequence of common carp MBSP and other serine proteinases

1. common carp MBSP; 2. crucian carp MBSP; 3. porcine trypsin; 4. bovine trypsin; 5. mekratin; 6. flounder trypsin

The molecular weight of carp MBSP as estimated from nucleotide sequence is 24.5 ku, which is approximately 5.5 ku smaller than that as judged from SDS-PAGE and gel-filtration[14]. Though the molecular weight estimated by SDS-PAGE and gel-filtration may not correctly show the size as the protein movement under electrophoresis could be affected by various factors such as the characteristic of gel and protein concentration, the possibility that common carp MBSP is a glycosylated protein should also be considered. However, we could not find any potential glycosylation site from the deduced amino acid sequence from Fig.3. Thus, whether MBSP is a glycosylated enzyme or not needs to be further clarified. This is the first report concerning the full-length sequence of a trypsin-type serine proteinase from fish myofibril. We believe our findings will help researchers in the study of similar proteinases both biochemically and genetically in the future.

References:

[1] Neurath H, Walsh KA. Role of proteolytic enzymes in biological regulation (a review)[J]. Proc Natl Acad Sci USA, 1976, 73:3825-3832.

[2] Fuller R S, Brake A, Thorner J. Yeast prohormone processing enzyme (Kex2 gene product) is a Ca^{2+}-dependent serine protease[J]. Proc Natl Acad Sci USA, 1989, 86:1434-1438.

[3] Thomas G, Thorne B A, Thomas L, *et al*. Yeast

Kex2 endopeptidase correctly cleaves a neuroendocrine prohormone in mammalian cells[J]. Science, 1988, 241:226 – 230.

[4] Zhou Y, Lindberg I. Purification and characterization of the prohormone convertase PC 1 (PC3)[J]. J Biol Chem, 1993, 268: 5615 – 5623.

[5] Nakayama K. Furin: a mammalian subtilisin/Kex2p-like endoprotease involved in processing of a wide variety of precursor proteins[J]. Biochem J, 1997, 327:625 – 635.

[6] Shen F S, Roberts S F, Lindberg I. A putative processing enzyme for proenkephalin in bovine adrenal chromaffin granule membranes[J]. J Biol Chem, 1989, 264:15600 – 15605.

[7] Tamanoue Y, Takahashi T, Takahashi K. Purification and characterization of two isoforms of serine proteinase from the microsomal membranes of rat liver[J]. J Biochem, 1993, 113:229 – 235

[8] Tsuchiya Y, Takahashi T, Sakurai Y, *et al*. Purification and characterization of a novel membrane-bound arginine-specific serine proteinase from porcine intestinal mucosa[J]. J Biol Chem, 1994, 269:32985 – 32991.

[9] Sangorrin M P, Martone C B, Sanchez J J. Myofibril-bound serine protease and its endogenous inhibitor in mouse: extraction, partial characterization and effect on myofibrils[J]. Comp Biochem Physiol, 2002, 131:713 – 723.

[10] Schiltz M, Fourme R, Broutin I, *et al*. The catalytic site of serine proteinases as a specific binding cavity for xenon[J]. Structure, 1995, 3:309 – 316.

[11] Yanagihara S, Nakaoka H, Hara K, *et al*. Purification and characterization of serine proteinase from white croaker skeletal muscle[J]. Bull Jan Soc Sci Fish, 1991, 57:133 – 142.

[12] Cao M J, Wu L L, Hara K, *et al*. Purification and characterization of a myofibril-bound serine proteinase (MBSP) from the skeletal muscle of silver carp[J]. J Food Biochem, 2005, 29:533 – 546.

[13] Cao M J, Jiang X J, Zhong H C, *et al*. Degradation of myofibrillar proteins by a myofibril-bound serine proteinase in the skeletal muscle of crucian carp[J]. Food Chem, 2006, 94:7 – 13.

[14] Osatomi K, Sasai H, Cao M J, *et al*. Purification and characterization of myofibril-bound serine proteinase from carp *Cyprius carpio* ordinary muscle[J]. Comp Biochem Physiol, 1997, 116B:159 – 166.

[15] Cao M J, Hara K, Osatomi K, *et al*. Myofibril-bound serine proteinase (MBP) and its degradation of myofibrillar proteins[J]. J Food Sci, 1999, 64:644 – 647.

[16] Cao M J, Osatomi K, Pangkey H, *et al*. Cleavage specificity of a myofibril-bound serine proteinase from carp (*Cyprinus carpio*) muscle[J]. Comp Biochem Physiol, 1999, 123:399 – 405.

[17] Hermodson M A, Ericsson L H, Neurath H, *et al*. Determination of the amino acid sequence of porcine trypsin by sequenator analysis[J]. Biochemistry, 1973, 12:3146 – 3153.

[18] Mikes O, Holeysovsky V, Tomasek V, *et al*. Covalent structure of bovine trypsinogen. The position of the remaining amides[J]. Biochem Biophys Res Commun, 1966, 24:346 – 352.

[19] Douglas S E, Gallant J W. Isolation of cDNAs for trypsinogen from the winter flounder, *Pleuronectes americanus*[J]. J Mar Biotechnol, 1998, 6:214 – 219.

[20] Holt J C, Hatcher V B, Caulfield J B, *et al*. Cloning of the cDNA and nucleotide sequence of a skeletal muscle protease from myopathic hamsters[J]. Mol Cell Biochem, 1998, 181:125 – 135.

鲤肌肉肌原纤维结合型丝氨酸蛋白酶的分子克隆

郭 川, 梁银龙, 刘光明, 苏文金, 曹敏杰
(集美大学生物工程学院,福建省高校水产养殖与食品安全重点实验室,福建 厦门 361021)

摘要:肌原纤维结合型丝氨酸蛋白酶(myofibril-bound serine proteinase, MBSP)是最近发现的一种蛋白酶。该酶参与肌原纤维蛋白的降解及鱼糜制品弹性的下降。但是,对该酶一级结构的研究,迄今为止,未有报道。本文根据已测定的鲤MBSP N-末端氨基酸序列以及丝氨酸蛋白酶活性中心保守序列设计兼并引物,结合RT-PCR技术实现了MBSP基因片段的扩增。再根据克隆到的MBSP片段序列设计基因特异引物,用于MBSP基因的5′和3′末端快速扩增。综合以上结果,鲤MBSP的全长被确定。序列分析表明,MBSP cDNA含有一732 bp的开放阅读框,编码243个氨基酸残基,其中信号肽长度为21个氨基酸残基。组成丝氨酸蛋白酶活性中心的氨基酸残基(His61, Asp107和Ser197)在MBSP中保守存在。成熟MBSP含有222个氨基酸残基,分子量为24.5 ku,比其天然蛋白的分子量30 ku略小。成熟MBSP的等电点为10.43。鲤MBSP与鲫MBSP,猪胰蛋白酶,牛胰蛋白酶,美洲鲽胰蛋白酶的同源性分别为80.6%,55.8%,55.3%和53.9%。而与仓鼠肌肉中具有胰凝乳蛋白酶性质的蛋白酶的同源性为39.2%。MBSP有高含量的赖氨酸残基(11.93%),此特性可能与该酶的肌原纤维结合特性有关。

关键词:鲤;克隆;肌原纤维结合型丝氨酸蛋白酶;同源性
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作者简介:郭 川(1981-),男,广东汕头人,硕士研究生,主要从事生物技术研究
通讯作者:曹敏杰,Tel:0592-6180378, E-mail: mjcao@jmu.edu.cn