



Molecular cloning and tissue distribution of hyaluronan binding protein 2 (HABP2) in red sea bream *Pagrus major*

Asami Yoshida^a, Yajun Wang^b, Inwoo Bae^c, Min-Jie Cao^d, Kiyoshi Osatomi^a, Kenji Hara^{a,*}

^a Graduate School of Fisheries Science and Environmental Studies, Nagasaki University, 1–14 Bunkyo, Nagasaki 852–8521, Japan

^b Department of Bacteriology, Graduate School of Medicine, Osaka City University, Osaka, 545–8585, Japan

^c Graduate School of Science and Technology, Nagasaki University, 1–14 Bunkyo, Nagasaki 852–8521, Japan

^d College of Biological Engineering, Jimei University, Jimei, Xiamen, Fujian 361021, China

ARTICLE INFO

Article history:

Received 25 April 2013

Received in revised form 24 May 2013

Accepted 24 May 2013

Available online 2 June 2013

Keywords:

Extracellular matrix proteolysis

Gelatinolytic serine proteinase

Hyaluronan binding protein 2 (HABP2)

Molecular cloning

Red sea bream

ABSTRACT

Previously we have isolated a novel gelatinolytic serine proteinase, named G1, from the muscle and the plasma of red sea bream. In order to clarify the structure and function of G1, we cloned the full-length cDNA of G1 from the hepatopancreas of red sea bream. G1 cDNA encoded 633 amino acids with a secretory signal sequence at N-terminus, three epidermal growth factor-like domains, a kringle domain, and a trypsin-like serine protease domain. The active site residues of a serine proteinase were conserved in the serine protease domain of G1. The tissue distributions of the mRNA and gelatinolytic activity of G1 were investigated using RT-PCR and gelatin zymography, respectively. Its activity was detected in various tissues while the mRNA of it was strongly expressed in the hepatopancreas. These results suggest that G1 is synthesized in hepatopancreas and carried to the muscle, kidney, heart and ovary via the bloodstream in the red sea bream. The enzyme has a similar domain structure and tissue distribution to those of human hyaluronan binding protein 2 (HABP2) engaged in the extracellular matrix (ECM) turnover. Thus, it is suggested that G1 is identified as HABP2 and is possibly involved in ECM proteolysis of red sea bream.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

The serine proteinases in blood contribute to not only the fibrinolysis and the blood coagulation but also extracellular matrix (ECM) turnover. The urokinase-type plasminogen activator (uPA)/plasminogen system, which is well known as the pericellular fibrinolysis cascade of serine proteinases in mammalian blood, is also considered to play an important role for pericellular ECM proteolysis through the activation of matrix metalloproteinases (MMPs) in various physiological and pathological processes (Murphy et al., 1992; Fukao et al., 1997; Mazzieri et al., 1997; Arai et al., 1998). On the cell surface, plasminogen is converted to plasmin on plasminogen receptor by uPA bound to uPA receptor on the same cell surface, and subsequently plasmin activates proMMPs such as pro-collagenase and pro-gelatinase. Finally, active collagenase (MMP-1) degrades type I collagen, and active gelatinase (MMP-2, MMP-9) degrades type IV collagen and gelatin (denatured collagens). As above, ECM proteolysis by serine proteinase or metalloproteinase has been extensively studied in mammals.

On the contrary, in fishes, there are few papers about ECM proteolysis particularly by serine proteinase. The existences of the collagenolytic serine proteinase (Wu et al., 2010) and the gelatinolytic serine proteinases (Lødemel et al., 2004; Wu et al., 2008; Yoshida et al., 2009a; Bae et al., 2010) in fish have been reported, however, their structure, function and relation to ECM proteolysis have not been clarified. Hence, the investigation on the structure and the function of fish serine proteinases is quite important to know how the ECM degradation system has changed through the evolutionary process from the viewpoint of the comparative biochemical study.

The research on the ECM proteolysis in fish muscle is also important for marine food science since fish value is usually influenced by post-mortem fish muscle softening, caused by the degradation of the structural muscle proteins such as myofibrillar or ECM proteins. It is generally known that the fish muscle protein degradation is caused by endogenous proteinases in the muscle (Kolodziejaska and Sikorski, 1996; Chéret et al., 2007).

In our previous study, a gelatinolytic serine proteinase named G1, which might be involved in the post-mortem fish muscle softening, was found in the skeletal muscle and the serum of red sea bream. The N-terminal amino acid sequence of purified G1 protein has been determined for 32 amino acid residues and compared with the sequences of the other serine proteinases (Yoshida et al., 2009b). Since the sequence showed high homology to hyaluronan binding protein 2 (HABP2) from human, it was presumed that G1

* Corresponding author. Tel./fax: +81 95 819 2828.

E-mail addresses: y-asami@nagasaki-u.ac.jp (A. Yoshida), y-wang@med.osaka-cu.ac.jp (Y. Wang), i17w00@yahoo.co.jp (I. Bae), mjcao@jmu.edu.cn (M.-J. Cao), osatomi@nagasaki-u.ac.jp (K. Osatomi), hara@nagasaki-u.ac.jp (K. Hara).

1	ACTCTTCTGGATGCTCTTCTGATCCACTGCCATC	<u>ATG</u> AACTTAAAGCTCCTCTTCGTTTGCCTCTTCTTAGCAGCGCTCGTCATACCTGCTGAACGAAA	99
1		M N L K L L F V C L F L A A L V I P A E L K	22
100	CCTAGGCACAAAGAACTTCATCATCGTGACCGTCACGATCATGTGCAACAGGAATGGGACATGGACTGGGACATCATAGACGGCAAGGCAAAATAT		198
23	P R H K K L H H R D R H D H V Q Q G M G H G L G H H R R G K A K Y		55
199	GACGACATAATTAAGATGTTTTTTTTGAGATCATAGATGTGGTTACCGGTGGTGATGATGATGAAGATGAAGAAAGTCATGCAGACTGGCTCTCT		297
56	D D I I K D V F F E I I D V V T G G D D D D E D E E S H A D W L S		88
298	GAACCTCAAGAGCCAGAAGGCAGATGCAACCAACCCCTTGCCCTCAACAATGGTGTGTGAGGAGAAAAGGGCAAAAAGGCCAAAATCAATGTGAT		396
89	E L Q E P E G R C N P N P C L N N G V C E E K K G K K G K I K C D		121
397	TGTCACAGACCTTTCAAGGGAAAGAGATGCGAGAAAGGTCCAAAACATGTTTCGAGAGGTAGATGTGGCGTGGTGAATGTGTCTGATTTCAACTCCT		495
122	C P R P F K G K R C E K G P K H C S R G R C G R G E C V L I S T P		154
496	CCGTTCTTTGAGTGCAAGTGCAAGGAGCCCTTCAGCCTCCACACTGCAGAACTGTTTCACTGTGTGAGCCTAACCCATGTAAGAATAATGAACATGC		594
155	P F F E C K C K E P F Q P P H C R T V S L C E P N P C K N N G T C		187
595	GTCAAGGATGGTAATGACTTTGACTGCCAGTGCCCTCTGGGGTACAGAGGACGTTTTCGCCATGTTGGCCAGATGACTGTACGTGGATGATGGAGAG		693
188	V K D G N D F D C Q C P L G Y R G R F C H V G P D D C Y V D D G E		220
694	TCATACCGTGGCAATGTGAGTGAGACAGATGATGGTCATGAATGCCCTTACTGGAACCTCACTTCATCTGAGCAGGAACCGATCCCTTTGACTCC		792
221	S Y R G N V S E T D D G H E C L Y W N S H F I L E Q G T D P F D S		253
793	TTGAGGACAAAGATGGACTTGGCCCTCACAACTTTCGAGAAACCCAGCAGGATGCGATGCCGTGGTGTTCCTTCAGAAGAGGCCGCAAGTTGTGTG		891
254	F E D K D G L G P H N F C R N P D G D A M P W C F F R R G R K L L		286
892	TGGGACTACTGTGATGTGACAGAGTGTCTGAACCAACAGGTGTGGGCCCACTGGTGTGTTCCTCCAGGCCCTGATCCCACTGCTCCAAAGCCCAA		990
287	W D Y C D V T E C P E P T G V A P T G V V P P G P D P T A P K P Q		319
991	CCTACATCTCAGCCTTCAACACCTCAACCCACAACAGTCCCAAGCCAGCGACCCGATACCCCAACAACAGAGAATCCAGCAGGCTCCACAA		1089
320	P T S S Q P S T P Q P T T V P K P A T T V P P T T E N P S Q A P Q		352
1090	CCTTCTTACCACCCCTGGTGTCTTCTGTCATTCAGTGCACCCCTCCATCACAACTGTTCTCCACCTGTGGGATGGCTCAGCAAAAAAACCCATT		1188
353	P S S T T P G A S V I P S A T P P S Q Q F S T C G M A Q P K K P I		385
1189	ACCCGAATCTTAGGTGGTCTGAAGGTCTCTCCCGGTCTATACCTGGCAGGTGTCCGTTCAAGTTAGACACAGAACTCCAACTGCCGTTCAAACAC		1287
386	T R I L G G L K V S P G S I P W Q V S V Q V R P Q N S N L P F K H		418
1288	ACGTGTGGAGGAGTTCTCATCGAGAGCTGTGGGTACTGACAGCTGGACTGCATTGAACCAACAAAGGACATGGAAGTGGCTATGGGAGGTCTGTCA		1386
419	T C G G V L I E S C W V L T A G H C I E P N K D M E V A M G G L S		451
1387	CTGAATATGGACAAACCCAGAGCAATCTAAGAGTTGAAGAGGCTATTAGACATGAGAACTACAGGAGACTCCTTCAGCTGTTTACACGACATA		1485
452	L N M D E P T E Q I L R V E E A I R H E N Y R E T P S A V Y N D I		484
1486	GGCTTGTGAGGCTGAATGGTACCAACGGAGTTTGTGCCATTGAGACGAGTTTGTGAAGACAGCCTGTCTGCCTGATGCTCAGCTGCCTGATGGGATC		1584
485	G L L R L N G T N G V C A I E T Q F V K T A C L P D A Q L P D G I		517
1585	GAGTGTAAAAATTTCTGATGGGGTGTCTGAGGAATTTCAATATGGTTCTAACCCTTGTGTGCGCCAAATGTACTGCTGATCAACAGGAAAAGTGC		1683
518	E C K I S G W G V T E E F Q Y G S N H L L S A N V L L I N Q E K C		550
1684	ATGGAGCCTGTGTGTTATGGCGCTGTCTGGATAATACTATGTTCTGTGCTGGCCACCTGCAGGAGGGGTGGATTCCTGCAGGGTGACTCTGGAGGA		1782
551	M E P V V Y G A V L D N T M F C A G H L Q G G V D S C Q G D S G G		583
1783	CCATTGACTTGAAGCAAAATGTACAGCGTTTGTATGGTATTGTGAGTTGGGAGACCAATGTGGAATGAAGAACAAGCCTGGGGTCTACACACGG		1881
584	P L T C K Q N G T S V V Y G I V S W G D Q C G M K N K P G V Y T R		616
1882	GTCACTACCTTCCTGGACTGGATCAAGTCAAGACTCAGGCAGCATCTCCATTAAGCAACCTTGATTACTCAGCAACATGCCAGAAAGTTTAGCCAATGA		1980
617	V T T F L D W I K S K T Q A A S P *		633
1981	CTTGTATTATGTACAGAGCGAGTATTACTCTTCCGTGATTAAGCTTTGGGATTTTGTGCACTTGTACCGGGTATTTTGTCTATCAGGTCATAACATAATT		2079
2080	TTTATGTTGAGATGCTACTTATTAACAACTCAAAAACAAAGCATTTTATAAAATGTTTGTCTTGTGTTTACATCTAAATGCCATGTTTCTTAGTCTT		2178
2179	TCCCTGAAGTGATACTTAAGCAATGGCAATCCAAAACCATATATTAATAATGATGCATGTATGGAAGGAAAATACACAAATAATTAACAGAAA		2277
2278	AAAAAAAAAAAAAA		2291

Fig. 1. Nucleotide and deduced amino acid sequences of G1 from red sea bream hepatopancreas. The N-terminal amino acid sequence of purified G1 is shaded in black and shown by white letters. Initial codon (ATG), stop codon (TAA), and putative poly-adenylation signal (AATAAA) are underlined. The active site residues of serine protease are indicated by boxes.

might have the same subunit structure as human HABP2. HABP2 was firstly found in human plasma (Choi-miura et al., 1996), and the synonyms are plasma hyaluronan binding protein, hepatocyte growth factor activator-like protein and factor VII-activating protease. It has been reported that human HABP2 contains three epidermal growth factor (EGF)-like domains, a kringle domain and a serine protease domain, and its mRNA is expressed in human liver (Choi-miura et al., 1996). Thus, we hypothesized that G1 would be generated in the hepatopancreas of red sea bream and have the same subunit structure and the function as HABP2. Therefore, in order to demonstrate the structure and function of G1, we have cloned its cDNA and have investigated the tissue distribution of its mRNA and enzyme activity.

2. Materials and methods

2.1. Fish

Cultured red sea bream (*Pagrus major*, Perciformes, Sparidae; body mass about 2 kg) were purchased from a culture farm in Nagasaki Prefecture, Japan. After the fish was decapitated, we collected hepatopancreas and other organs for RNA isolation and measurement of protease activity.

2.2. Total RNA isolation, cDNA synthesis and RACE

The fresh hepatopancreas from the red sea bream was immediately soaked in 5 volumes of RNAlater (Ambion, Austin, TX, USA) to stabilize

Download English Version:

<https://daneshyari.com/en/article/1975280>

Download Persian Version:

<https://daneshyari.com/article/1975280>

[Daneshyari.com](https://daneshyari.com)