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

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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.

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 (Kolodziejska 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

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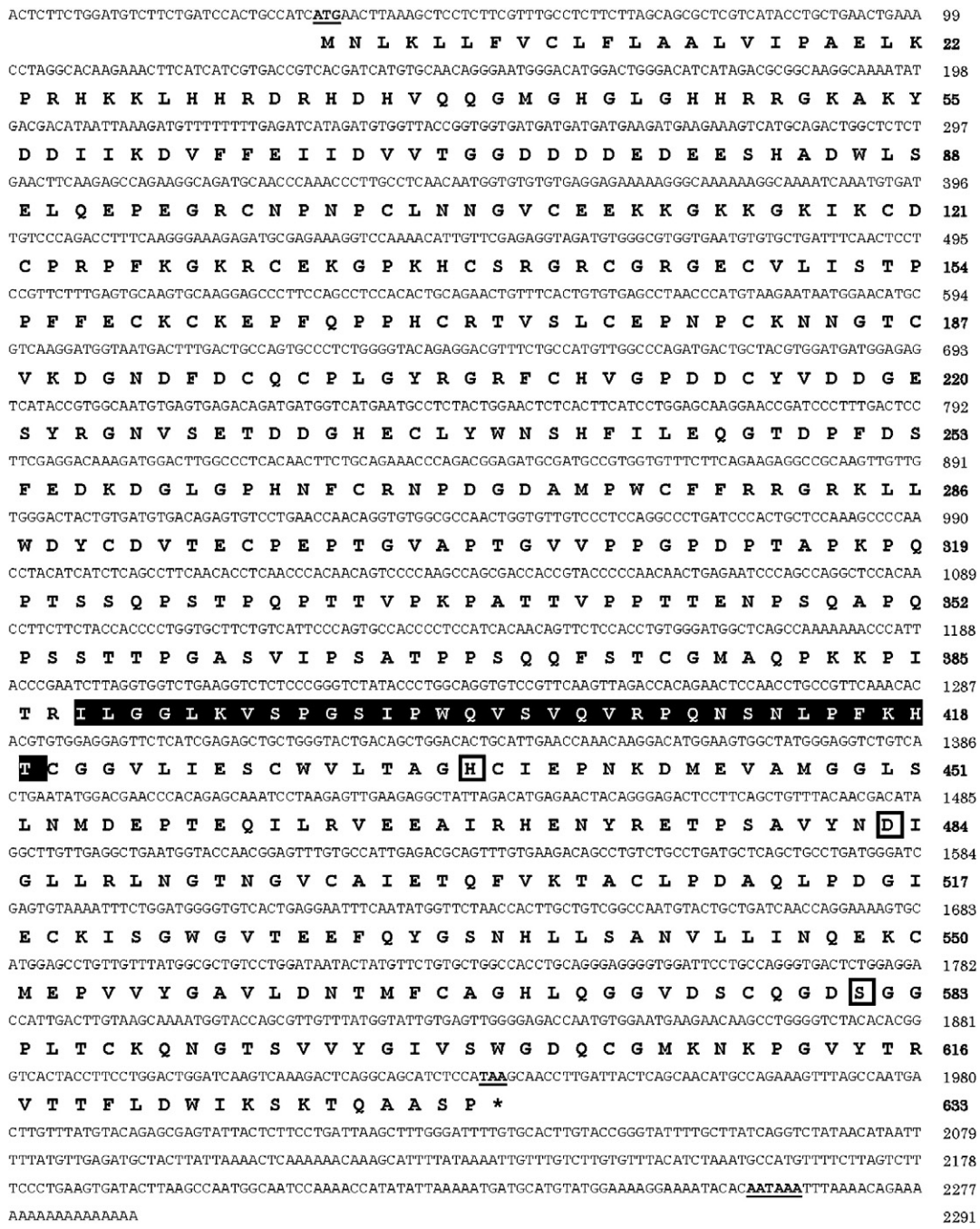

Fig. 1. Nucleotide and deduced amino acid sequences of G1 from red sea bream hepatopancreas. The N-terminal amino acid sequence of purified G1 is shaded in black and shown by white letters. Initial codon (ATG), stop codon (TAA), and putative poly-adenylation signal (AATAAA) are underlined. The active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