

A Kunitz-Type Serine Protease Inhibitor from Rock Bream, *Oplegnathus fasciatus* Involved in Immune Responses

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Abstract : Kunitz-type serine protease inhibitors (KTIs) are identified in various organisms including animals, plants and microbes. These proteins shared single or multiple Kunitz inhibitory domains link together or associated with other types of domains. Characteristic Kunitz type domain composed of around 60 amino acid residues with six conserved cysteine residues to stabilize by three disulfide bridges. KTIs are involved in various physiological processes, such as ion channel blocking, blood coagulation, fibrinolysis and inflammation. In this study, two Kunitz-type domain containing protein was identified from rock bream database and designated as RbKunitz. The coding sequence of RbKunitz encoded for 507 amino acids with 56.2 kDa theoretical molecular mass and 5.7 isoelectric point (pI). There are several functional domains including MANEC superfamily domain, PKD superfamily domain, and LDLa domain were predicted in addition to the two characteristic Kunitz domain. Moreover, trypsin interaction sites were also identified in Kunitz domain. Homology analysis revealed that RbKunitz shared highest identity (77.6%) with Takifugu rubripes. Completely conserved 28 cysteine residues were recognized, when comparison of RbKunitz with other orthologs from different taxonomical groups. These structural evidences indicate the rigidity of RbKunitz folding structure to achieve the proper function. The phylogenetic tree was constructed using neighbor-joining method and exhibited that the KTIs from fish and non-fish has been evolved in separately. Rock bream was clustered with Takifugu rubripes. The SYBR Green qPCR was performed to quantify the RbKunitz transcripts in different tissues and challenged tissues. The mRNA transcripts of RbKunitz were detected in all tissues (muscle, spleen, head kidney, blood, heart, skin, liver, intestine, kidney and gills) analyzed and highest transcripts level was detected in gill tissues. Temporal transcription profile of RbKunitz in rock bream blood tissues was analyzed upon LPS (lipopolysaccharide), Poly I:C (Polyinosinic:polycytidylic acid) and *Edwardsiella tarda* challenge to understand the immune responses of this gene. Compare to the unchallenged control RbKunitz exhibited strong up-regulation at 24 h post injection (p.i.) after LPS and *E. tarda* injection. Comparatively robust expression of RbKunits was observed at 3 h p.i. upon Poly I:C challenge. Taken together all these data indicate that RbKunitz may involve into to immune responses upon pathogenic stress, in order to protect the rock bream.

Keywords : Kunitz-type, rock bream, immune response, serine protease inhibitor

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