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Cloning and expression analysis of an *O*-methyltransferase (OMT) gene from Chinese shrimp, *Fenneropenaeus chinensis*☆

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Abstract

O-methyltransferase (OMT) is ubiquitously present in diverse organisms and plays an important regulatory role in plant and animal growth, development, reproduction and defence and has also been implicated in human emotion and disease. A putative *O*-methyltransferase (OMT) gene has been cloned from the haemocytes of bacteria-infected Chinese shrimp (*Fenneropenaeus chinensis*) by suppression subtractive hybridisation (SSH) coupled with the SMART cDNA method. The isolated 944 bp full-length cDNA contains a single 666 bp open reading frame (ORF) encoding a putative OMT protein of 221 amino acids. The predicted protein has a molecular weight of 24 572.06 Da and a p*I* of 5.27 as well as ten phosphorylation sites. Northern blot and in situ hybridisation analyses demonstrated that the OMT transcripts were constitutively expressed in tissue of shrimp challenged by bacterial infection and in unchallenged shrimp tissue. Constitutive OMT transcript was found in areas such as haemocytes, heart, hepatopancreas, stomach, gill, intestine and ovary. However, the OMT transcripts were upregulated in hepatopancreas and stomach in challenged shrimp.

Keywords: *O*-Methyltransferase; *Fenneropenaeus chinensis*; Gene cloning; Expression pattern

1. Introduction

O-methyltransferase (OMT) is an enzyme that is ubiquitously present across diverse organisms including bacteria [1], fungi [2], plants [3] and animals [4]. It catalyses the methylation of both small and macromolecules for various functional and regulatory purposes. OMT has been implicated in the regulation of both plant and animal growth, development, and reproduction [5,6]. It is involved in plant defence systems and interactions with environmental factors [7]. Additionally, there is evidence suggesting that OMT may play a role in human emotion and disease [8,9].

Although the chemical mechanisms of methyl transfer reactions are identical, *O*-methyltransferases differ in their selectivity to methyl acceptor molecules. In animals, there are two kinds of *O*-methyltransferases studied. Studies of crustacean farnesoic acid *O*-methyltransferase (FAMeT) [10–13] indicate that FAMeT catalyses the methylation of

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farnesoic acid (FA) to produce isoprenoid methyl farnesoate (MF) at the final step of the MF biosynthetic pathway. Due to the structural similarities of MF and insect juvenile hormone (JH III), it has been suggested that MF acts as a juvenile hormone in crustacea. Thus, MF may regulate crustacean growth, reproduction, ovarian development and molting. The other *O*-methyltransferase studied in animals, catechol-*O*-methyltransferase (COMT), can catalyse the transfer of the methyl group from *S*-adenosine-methionine to the hydroxyl group of catechol compounds such as catecholamines. Thus, COMT plays an important role in the catabolism and *O*-methylation of endogenous catecholamines, such as dopamine and noradrenaline, in the brains of animals [14].

To date, several crustacean FAMeT sequences have been published in on-line databases, including shrimp *Metapenaeus ensis* FAMeT [15], American lobster *Homarus americanus* FAMeT [16], crab *Cancer pagurus* FAMeT [10] and *Panulirus interruptus* FAMeT (GenBank accession number AAF65551). However, no COMT sequence from crustaceans has been described. Although some plant *O*-methyltransferases have been involved in immunoreactions [17,18], the role of animal *O*-methyltransferases in immunity remains unknown. In this study, using suppression subtractive hybridisation (SSH) and SMART cDNA methods, the putative *O*-methyltransferase gene from Chinese shrimp *F. chinensis* was cloned. Moreover, the characterisation and tissue distribution of the OMT gene is described.

2. Materials and methods

2.1. Animals, tissues and haemolymph

Adult *Fenneropenaeus chinensis* (Crustacea, Decapoda) were purchased from a market in Qingdao city in the Shandong province of China, and maintained in an oxygenated, recirculating and ambient seawater system. Challenged shrimp were injected with a mixture of live *Vibrio anguillarum* and *Staphylococcus aureus* (with 3×10^6 cfu). After being in culture for 24 h, haemolymph of shrimp from challenged and control groups was collected according to the method of Destoumieux and co-workers [19]. The haemolymph was immediately centrifuged at 700G for 15 min (4 °C) to separate the haemocytes from plasma. After haemolymph collection, shrimp were dissected. Tissue samples were pooled from each group, frozen in liquid nitrogen, and stored at −80 °C.

2.2. Construction of subtracted cDNA library

mRNA was extracted from the haemocytes of challenged and unchallenged shrimp using QuickPrep, a micro mRNA Purification Kit (Amersham Biosciences). cDNA was synthesised, according to the manufacturer's instructions, using a Super SMART-PCR cDNA Synthesis Kit. Finally, the subtracted cDNA library was constructed following the instructions of a Clontech PCR-Select™ cDNA subtraction Kit (Clontech). Briefly, challenged and control cDNA populations were separately digested by *Rsa*I. The ''tester'' *Rsa*I-digested cDNA population from the challenged group, was divided into two pools. Each pool interacted with adaptor 1 and adaptor 2R, provided by the kit, whereas the *Rsa*I-digested cDNA population of the ''driver'', or control group, was not exposed to adaptors. After two hybridisations, the resulting mixture was diluted to 1:50 and then amplified by two rounds of PCR to enrich desired cDNAs containing both adaptors by exponential amplification of these products.

2.3. Screening of subtracted cDNA library

The PCR product from secondary PCR amplification of SSH was directly inserted into pGEM-T Easy vector (Promega), then transformed into *E. coli* DH5α cells, and plated onto LB medium with ampicillin, X-gal, and IPTG and cultured overnight. White clones were picked out randomly and the insert cDNAs of the clones were assayed by PCR with the vector primers (T7 and SP6) and sequenced by Sangon Company. A 371 bp cDNA clone was identified as the 3′ end of putative *O*-methyltransferase by searching using BLASTx at NCBI (http://www.ncbi.nlm.nih.gov/blast).

2.4. Gene cloning

The 371 bp cDNA fragment provided a gene-specific primer (Meth R1, 5′ GTGATCGACCCCACTGATCAA3′). Using the 5′ SMART and R1 primers and the SMART cDNA template that was synthesised from challenged