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Molecular Cloning and Homology Modeling of Novel Tyrosylprotein Sulfotransferase of Marine Mollusk

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ABSTRACT

The gene of tyrosylprotein sulfotransferase, which was discovered in mammals, has been widely found in marine mollusk *Littorina sitkana*. High conservation of this gene indicates the functional importance of TPST in the metabolism of the living world. The cDNA encoding TPST in the mollusk was cloned and sequenced, and the enzyme was assigned on the basis of amino acid sequence similarity as tyrosylprotein sulfotransferase-2 (TPST-2). The putative homology model for the catalytic domain of TPST from *L. sitkana* was constructed according to crystal structure of the catalytic domain of the human TPST-2. The putative model of dimer structure showed that the active site involved two monomers and the dimer contains two active centers.

Introduction

Tyrosylprotein sulfotransferases (TPSTs[1], EC 2.8.2.20) are the Golgi-localized type II transmembrane proteins, which transfer a sulfuryl group (SO_3^{2-}) from the universal sulfate donor 3′-phosphoadenosine-5′-phosphosulfate (PAPS) to the hydroxyl of tyrosine side chain (Lee and Huttner, 1983). Tyrosine sulfation is a common post-translational modification of peptides and proteins. TPST activity has been described in many species of animals and plants (Niehrs and Huttner, 1990; Sane and Baker, 1993; Kasinathan et al., 2005; Nishimura and Naito, 2007; Hanai et al., 2000). In 2012, this enzyme was first discovered in a Gram-negative bacterium (Han et al., 2012), although tyrosine sulfation has not previously been reported in prokaryotes. In spite of the functional importance of these enzymes, almost all known TPSTs are of mammalian origins. Only few enzymes from invertebrates have been described (from nematodes *Caenorhabditis elegans* and *Brugia malayi*, insects *Culex quinquefasciatus*, *Drosophila* spp., and *Anopheles gambiae*, sea squirts *Ciona intestinalis* and *Halocynthia roretzi*, trematode *Schistosoma japonicum*, marine mollusk *Crassostrea gigas*) mainly via cDNA or genome sequencing.

The sulfation of biomolecules plays an important role in the metabolism of pro- and eukaryotes. The growing scientific interest in the sulfation of different natural compounds stems from the high biological activities of sulfated derivatives and their role in organisms. The known roles of tyrosine sulfation in mammals are various and multiple, for example maintenance of hemostasis,

Abbreviations: TPST, tyrosylprotein sulfotransferases; PAPS, 3′-phosphoadenosine-5′-phosphosulfate; LsTPST, tyrosylprotein sulfotransferases from *Littorina sitkana*.

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triggering inflammatory responses, strengthening leucocyte adhesion, defining specificity of chemokine receptors and enhancement of potency of bioactive peptides (Coughtrie et al., 1998; Kehoe and Bertozzi, 2000; Moore, 2003; Seibert and Sakmar, 2008).

Marine hydrobionts are extremely rich in sulfated metabolites with diverse biological activities (Kornprobst et al., 1998). Unfortunately, sulfation/desulfation processes are studied considerably less than, for example, phosphorylation. The knowledge about the proteins undergoing sulfation in marine organisms is particularly poor. Frequently enzymes from marine habitats have unique properties; some of them are very useful for biotechnological applications. The enzymes with interesting specificities and high level of activities were found in the digestive glands of *Littorina sitkana*. Previously we isolated and characterized from this mollusk the enzymes, which catalyze hydrolysis and transformation of carbohydrate-containing natural products. There are alginate lyase (Favorov et al., 1979), two forms of fucoidanases (Kusaykin et al., 2003; Bilan et al., 2005), endo-1,3-β-D-glucanase (Pesentseva et al., 2012), β-D-glucosidase (Pesentseva et al., 2008) and sulfatase (Kusaykin et al., 2006). Herbivorous marine gastropod *L. sitkana* is widely spread on the coasts of the Pacific and the Atlantic Oceans, and it was interesting to search the TPST gene in this animal. The data of the crystal structure of human tyrosylprotein sulfotransferase-2 (Teramoto et al., 2013) and the homology model for human TPST-1 (Nedumpully-Govindan et al., 2014) will give more information about this enzyme.

Thus, the present study was devoted to the search for the tyrosylprotein sulfotransferase gene in the marine gastropod, cloning and sequencing of the cDNA encoding this protein, and 3D-structure homology modeling.

Materials and Methods

Biological Material

Marine mollusks *L. sitkana* were collected in the Posieta Bay (northwestern part of the Sea of Japan) in August 2014 near the Marine Experimental Station of the Pacific Institute of Bioorganic Chemistry.

RNA Isolation and cDNA Synthesis

Total RNA was isolated from the liver of *L. sitkana* by the TRIzol Reagent (Invitrogen, USA) and cDNA was synthesized with Mint cDNA Synthesis Kit (Eurogen, Russia) according to the provided protocols.

Amplification of TPST cDNA

The fragments of cDNA of TPST from *L. sitkana* were obtained by PCR using cDNA from *L. sitkana*. The SU-F1, SU-F2, SU-R2, SU-R3, and SU-R4 primers (Table 1) synthesized on the basis of the conserved peptides were used for the amplification, which was carried out for 35 cycles (10 s at 95 °C, 15 s at 55 °C, 40 s at 72 °C). The terminal cDNA regions were obtained by rapid amplification of the cDNA fragments (RACE). The amplification was performed with SU-5race and SU-3race primers for 38 cycles (10 s at 95 °C, 20 s at 63 °C, 60 s at 72 °C).

Cloning and Sequencing of cDNA of TPST From L. sitkana

The PCR products were cloned with InsTAclone PCR Cloning Kit from Fermentas (Lithuania) according to the manufacturer's recommendations. Bacterial colonies containing plasmids with the desired insertion were screened by PCR using M13 universal primers. Nucleotide sequences were determined with ABI Prism Big Dye Terminator 3.1 Cycle Sequencing Kit from Applied Biosystems (USA) on ABI Prism 310 Genetic Analyzer.

Analysis of Nucleotide and Amino Acid Sequences

The nucleotide and amino acid sequences were analyzed using the programs CHROMAS 2.01 (http://www.technelysium.com.au/chromas_lite.html) and GENERUNNER 3.05. Amino acid sequence was established on the basis of the nucleotide sequence of the cDNA encoding TPST with EXPASY (http://expasy.org/tools/dna.html). Search of TPST homologous was performed using the BLAST2 (http://www.ebi.ac.uk/blastall). Domain architectures were identified by SMART tool (http://smart.embl-heidelberg.de)

Table 1
Primers for amplification of TPST cDNA of *L. sitkana*.

No.	Primer	Nucleotide sequence
1	SU-F1	5′-ATTTTTATTGGIGGIGTICCICG-3′
2	SU-F2	5′-ATGCTTGATGCTCATCCTGATGT-3′
3	SU-R2	5′-ATTAACAGGTTTAATAACTTGATCAGT-3′
4	SU-R3	5′-ATCAGGTTTACCATAATTAGGAGGATT-3′
5	SU-R4	5′-ATCAGGTTTACCATAATTAGGAGGATT-3′
6	SU-5race	5′-TTCCGGTAGCTCTTGAGGT-3′
7	SU-3race	5′-GACGGAGAAGTCGACGGAC-3′