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Mast cell is one of the most important cells in immune systems and contains a variety of proteases (tryptase, chymase and carboxypeptidase-A), heparin etc in their secretory granules. During inflammation mast cells release those proteases from their granules. Therefore, knowledge of the regulation of mast cell proteases and their interactions with heparin will offer enormous therapeutic potentials in the treatment of a number of diseases and conditions such as cardiovascular, arthritic, toxin clearance, and removal from infectious diseases etc.

Heparin/heparan sulfate (HS) proteoglycans are composed of sulfated polysaccharides attached to the core protein. The backbone polysaccharides of HS/heparin are synthesized by the alternating addition GlcUA and GlcNAc residues from their respective UDP-sugar precursors, and are partially modified by a number of enzymes such as C-5 epimerase, *N*-deacetylase/*N*-sulfotransferase, 2-*O*-, 6-*O*- and 3-*O*-sulfotransferases.

Heparan sulfate 6-*O*-sulfotransferase (HS6ST) is an enzyme involved in heparan sulfate (HS) biosynthesis that transfers a sulfate residue to position 6 of the GlcNAc/$GlcNSO_3$ residues of HS, and it consists of three isoforms (HS6ST-1, -2, -3). Heparin, the highly sulfated form of HS, resides in connective tissue mast cells and is involved in the storage of mast cell proteases (MCPs) in the granules. However, it is not well understood which isoform(s) of HS6ST participates in 6-*O*-sulfation of heparin and how the 6-*O*-sulfate residues in heparin affect MCPs. To investigate these questions, we prepared fetal skin-derived mast cells (FSMCs) from wild-type (WT) and HS6ST-deficient mice (HS6ST-$1^{-/-}$, HS6ST-$2^{-/-}$, and HS6ST-$1^{-/-}$/HS6ST-$2^{-/-}$) and determined the structure of heparin, the protease activity, and the mRNA expression of each MCP in cultured FSMCs. The activities of tryptase and carboxypeptidase-A (CPA) were decreased in HS6ST-$2^{-/-}$-FSMCs in which 6-*O*-sulfation of heparin was decreased at 50% of WT-FSMCs, and almost lost in HS6ST-$1^{-/-}$/HS6ST-$2^{-/-}$-FSMCs which lacked the 6-*O*-sulfation in heparin nearly completely (Figure-1 and Table-1). In contrast, chymase activity was retained even in HS6ST-$1^{-/-}$/HS6ST-$2^{-/-}$-FSMCs (Table-1). Each MCP mRNA was not decreased in any of the mutant FSMCs (Figure-2). Western blot analysis showed that tryptase (mMCP-6) was almost absent from HS6ST-$1^{-/-}$/HS6ST-$2^{-/-}$-FSMCs (Figure-3) indicating degradation/secretion of the enzyme protein. These observations suggest that both HS6ST-1 and HS6ST-2 are involved in 6-*O*-sulfation of heparin and that the proper packaging and storage of tryptase, CPA, and chymase with heparin in the granules may be regulated differently by the 6-*O*-sulfate residues in heparin. It is thus likely that 6-*O*-sulfation of heparin plays important roles in regulating MCP functions.

The research bears immense clinical significance in autoimmune diseases such as autoimmune arthritis. Mast cell tryptase was found to have attenuated arthritic response via tryptase-heparin complexes in tryptase-KO animals that developed lower inflammation and bone/cartilage erosion than did WT mice. Therefore, it is possible that HS6ST activity might be involved in the specific regulation of some protease activities in CTMCs. For example, a possible up-regulation of HS6ST activity in patients during arthritic inflammation might affect the treatment of this disease. Therefore, some reagents that are able to regulate HS-6-*O*-sulfation might have therapeutic potential in diseases involving mast cell proteases.

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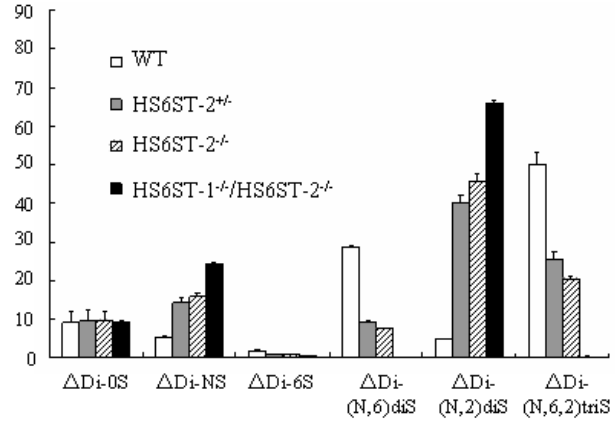
Figure-1. Disaccharide composition analysis of HS/heparin from WT and HS6ST-2$^{+/-}$, HS6ST-2$^{-/-}$, and HS6ST-1$^{-/-}$HS6ST-2$^{-/-}$ fetal skin-derived mast cells (FSMCs).



Glycosaminoglycans (GAGs) from WT and mutant FSMCs were isolated and analyzed. The histograms show the percentage composition of the unsaturated disaccharides in HS/heparin from wild type (*open bar*) and HS6ST-2$^{+/-}$ (*grey bar*), HS6ST-2$^{-/-}$ (*hatch bar*), and HS6ST-1$^{-/-}$HS6ST-2$^{-/-}$ HS6ST-1$^{-/-}$-FSMCs (*filled bar*).

Table-1. Relative activities of MCPs in WT, HS6ST2$^{+/-}$, HS6ST2$^{-/-}$, and HS6ST-1$^{-/-}$/HS6ST-2$^{-/-}$-FSMCs. Activities were determined from the linear portion of the curve and normalized as per microgram of protein of the cell extract from each cell type.

FSMC	Relative activity		
	Tryptase	Chymase	CPA
WT	1	1	1
HS6ST2$^{+/-}$	0.37 ± 0.09	0.68 ± 0.02	0.74 ± 0.01
HS6ST2$^{-/-}$	0.29 ± 0.07	0.56 ± 0.06	0.51 ± 0.025
HS6ST-1$^{-/-}$/HS6ST-2$^{-/-}$	≤0.05	0.46 ± 0.01	≤0.03

Figure-2 Expression levels of mast cell proteases in WT, HS6ST2$^{+/-}$, HS6ST2$^{-/-}$, and HS6ST-1$^{-/-}$/HS6ST-2$^{-/-}$-FSMCs. Total RNA was isolated and were measured by semi-quantitative RT-PCR using specific primers. The expression level of β–actin was used as a control.

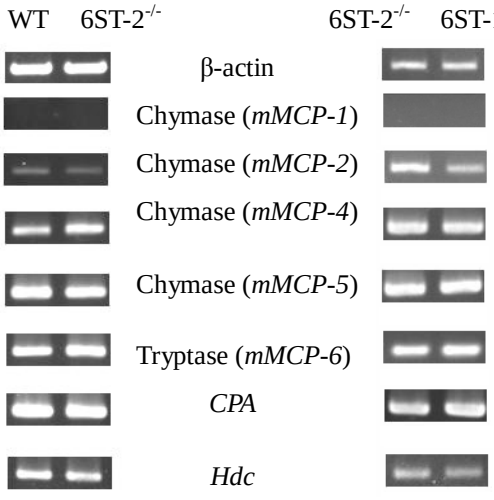


Figure-3. Immunoblot analysis of tryptase (mMCP-6) from HS6ST-1$^{-/-}$/HS6ST-2$^{-/-}$-FSMCS