Characterization of a Heparan Sulfate 3-O-Sulfotransferase-5, an Enzyme Synthesizing a Tetrasulfated Disaccharide*

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Heparan sulfate 3-glucosaminyl 3-O-sulfotransferases (3-OSTs) catalyze the transfer of sulfate from 3'-phosphoadenosine 5'-phosphosulfate (PAPS) to position 3 of the glucosamine residue of heparan sulfate and heparin. A sixth member of the human 3-OST family, named 3-OST-5, was recently reported (Xia, G., Chen, J., Tiwari, V., Ju, W., Li, J.-P., Malmstrom, A., Shukla, D., and Liu, J. (2002) J. Biol. Chem. 277, 37912-37919). In the present study, we cloned putative catalytic domain of the human 3-OST-5 and expressed it in insect cells as a soluble enzyme. Recombinant 3-OST-5 only exhibited sulfotransferase activity toward heparan sulfate and heparin. When incubated heparin sulfate with [35S]PAPS, the highest incorporation of [35S]PAPS was observed, and digestion of the product with a mixture of heparin lyases yielded two major, [35S]PAPS-labeled disaccharides, which were determined as ΔHexA-GlcNS(3S,5S,8S) and ΔHexA(2S)-GlcNS(3S,5S,8S) by further digestion with 2-sulfatase and degradation with mercuric acetate. However, when used heparin as acceptor, we identified a highly sulfated disaccharide unit but also the enzymatic formation of this novel structure.

Heparan sulfate proteoglycans (HSPGs) are ubiquitously present on the cell surface and in the extracellular matrix and have divergent structures and functions (1-3). Many biological functions of HSPGs are mediated by interactions between the heparan sulfate chain and a variety of proteins, including protease inhibitors, heparin-binding growth factors, extracellular matrix components, protease, and lipoprotein lipase (4-8). Moreover, some pathogens exploit heparan sulfate of the host cell surface, which binds to coat proteins or cell surface proteins of pathogens, at the invasion (9, 10). Most of the interactions between heparan sulfate and various functional proteins occur in certain regions of the heparan sulfate chain with specific sulfated monosaccharide sequences such as binding sites for antithrombin III (AT III) (11), acidic fibroblast growth factor (12), basic fibroblast growth factor (13-16), and hepatocyte growth factor (17, 18). The ability of cells to produce heparan sulfate with such sequences depends on the specific mechanisms of heparan sulfate biosynthesis (19, 20). Heparan sulfate is initially synthesized as a polymer of a disaccharide repeat sequence, -glucuronic acid-β1,4-N-acetylgalactosamine-α1,4-... This polymer is then N-deacetylated/N-sulfated and subsequently undergoes epimerization of glucuronic acid (GlcA) to iduronic acid (IdoA), 2-O-sulfation of uronic acid, and 6-O-sulfation of glucosamine residues. Additionally, a rare but functionally important modification, 3-O-sulfation of the glucosamine residue, also occurs (21). Most of the enzymes involved in the biosynthesis of heparan sulfate have been purified and cloned (22, 23). Some of them have been shown to be present as isoforms. These isoforms play important roles in generating the specific and diverse structures of heparan sulfate. Four isoforms of N-deacetylase/N-sulfotransferase have been reported.

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1 The abbreviations used are: HSPG, heparan sulfate proteoglycan; AT III, antithrombin III; gD, glycoprotein D; 3-OST, heparan sulfate 3-glucosaminyl 3-O-sulfotransferase; HSV-1, herpes simplex virus type 1; GlcA, D-glucuronic acid; IdoA, L-iduronic acid; GlcN, D-glucosamine; GlcNAc, N-acetyl-D-glucosamine; αMan, 2,3-anhydro-D-mannitol; ΔHexA, 3,5-diunsaturated uronic acid; 2S, 2-O-sulfate; NS, 2-N-sulfate; 3S, 3-O-sulfate; 6S, 6-O-sulfate; ΔDi-6S, ΔHexA-GlcNAc; ΔDi-NS, ΔHexA-GlcNS(NS); ΔDi-8S, ΔHexA-GlcNAc(NS,8S); ΔDi-diS1, ΔHexA-GlcNS(NS,8S); ΔDi-diS2, ΔHexA(2S)-GlcNS(NS); ΔDi-triS1, ΔHexA(2S)-GlcNS(NS,8S); ΔDi-triS2, ΔHexA-GlcNS(NS,8S,3S,6S); ΔDi-triS3, ΔHexA(2S)-GlcNS(NS,8S,3S); ΔDi-tetaS, ΔHexA(2S)-GlcNS(NS,8S,3S,6S); PAPS, 3′-phosphoadenosine 5′-phosphosulfate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; SAX, strong anion-exchange; HPLC, high performance liquid chromatography.

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These isoforms are distinguishable from each other with respect to the extent of N-sulfation of heparan sulfate, the ratio of N-deacetylasparagine to N-sulfotransferase activities and the expression pattern in various tissues (24–26). Three isoforms of heparan sulfate 6-O-sulfotransferase were found to have different specificities and different expression patterns (27). Six isoforms of 3-O-sulfotransferase (3-OST) with different spectra of acceptor substrate specificity have also been reported. 3-OST-1 transfers sulfates to GlcN-GlcNS,7,6S and produces the GlcN-GlcNS,3S,5S units. The putative catalytic domain of 3-OST-5 (amino acids 63 to 175) was cloned and expressed as a secreted protein fused with a FLAG Peptide—

The secreted enzyme was purified using anti-FLAG M1 monoclonal antibody agarose affinity gel (Sigma). The culture media and affinity gel were mixed overnight at 4 °C and centrifuged for 5 min, and the supernatant was aspirated. The affinity gel was washed twice with 50 mM Tris-HCl, pH 7.4, and 150 mM NaCl containing 1 mM CaCl₂, and resuspended in 50 mM TBS to obtain a 50% slurry. The immobilized enzyme was stable at ~80 °C for at least six months.

**Assay for Sulfotransferase Activity**—The standard reaction mixture contained 50 mM imidazole-HCl, pH 6.8, 75 μM of p-bromotoluene, 0.5 μM (as hexosamine) acceptor substrates, 1.5 μM [35S]PAPS (about 2 × 10⁸ dpm), and 1 μl of immobilized enzyme in a 50 μl assay. Acceptor substrates used to examine the substrate specificity were heparan sulfate, heparin, chondroitin sulfate A, chondroitin sulfate C, dermatan sulfate, keratan sulfate, and hyaluronic acid. After incubation at 37°C for 20 min, the reaction was stopped by heating at 100°C for 1 min. The reaction mixture was filtered with an Ultralense-MC (MILLIPORE, Bedford, MA) and 60 μg of chondroitin sulfate C was added as a carrier. 35S-Labeled substrates were precipitated with 3 volumes of ethanol containing 1.3% potassium acetate and separated completely from [35S]PAPS and its degradation products by gel filtration HPLC on a Tosoh G2500PW column (0.7 × 30 cm) equilibrated with 0.2 mM NaCl at a flow rate of 0.6 ml/min and the column temperature was 25°C. Fractions of 0.5 ml were collected and the radioactivity was determined by liquid scintillation counting.

**Preparation of Oligosaccharides from Heparin**—It was reported that the heparinase digestion of heparin produces about 45% each disaccharide and tetrascarabide, and a few percent hexascarabide (36, 37). Two milligrams of heparin was digested with 0.2 units of heparinase and applied to a BioGel P-4 column (1.5 × 140 cm) equilibrated with 0.1 M ammonium bicarbonate. Elution positions of each oligosaccharide were monitored at 232 nm. Tetrascarabide and hexascarabide fractions were pooled and desalted by lyophilization.

**HPLC Analysis on an Anion-exchange Column and a Gel Filtration Column**—Anion-exchange (SAX) separation was performed on a Carbopac PA1 column (4 × 250 mm) as described in Materials and Methods. A combination of five linear LiCl gradients was used, from 30 to 180 mM (5–8 min), from 180 to 570 mM (5–8 min), from 570 to 1144 mM (8–15 min), from 1144 to 2288 mM (15–20 min) and from 2288 to 4488 mM (20–28 min) followed by 2.28 M (from 28 min) followed by 2.28 M (from 26 min). The flow rate was 0.8 ml/min, and the column temperature was 45°C. Fractions were collected and the radioactivity was determined by liquid scintillation counting. To determine the molecular size of oligosaccharides, gel filtration HPLC was performed on a Superdex™ Peptide HR10/30 column (1 × 30 cm, 2 columns in series) as described (39). The column was equilibrated with 0.2 M NaCl at a flow rate of 0.8 ml/min, at room temperature.

**Preparation of [35S]-Labeled Heparan Sulfate and Digestion with Heparinase**—One milligram of heparan sulfate from bovine kidney and [35S]PAPS (6.8 × 10⁷ dpm) was incubated with 0.5 ml of standard reaction mixture containing 67 μl of immobilized enzyme at 37°C for 3 h. After the incubation, the reaction mixture was filtered, and [35S]-labeled heparan sulfate was precipitated with ethanol as described above. The precipitate was dried at room temperature. The [35S]-labeled heparan sulfate was digested with a mixture of 0.5 units of heparinase, 0.3 units of heparitinase I, and 0.2 units of heparitinase II in 0.3 ml of 20 mM sodium acetate buffer (pH 7.0) containing 2 mM calcium acetate at 37°C for 2 h. The reaction was stopped by heating at 100°C for 1 min, and the mixture was filtered. The digested products were subjected to chromatography on a BioGel P-4 column (1.5 × 140 cm) equilibrated with 50 mM sodium acetate buffer (pH 6.7) at a flow rate of 0.8 ml/min. Fractions of 2 ml were collected. The fractions indicated by horizontal bars in Fig. 2, named P4-1 and P4-2, were pooled, lyophilized and completely desalted with a gel filtration column.

**Digestion with 2-Sulfatase**—The [35S]-labeled disaccharides were digested with 4 μg of 2-sulfatase in 20 mM sodium acetate buffer (pH 7.0) containing 1.5 mg/ml bovine serum albumin (BSA) and incubated at 37°C for 2 h, the reaction was stopped by heating at 100°C for 1 min. The digested products were analyzed by HPLC on a SAX column as described above.

**Determination of Glucosamine Residue**—The [35S]-labeled disaccharides were treated with mercuric acetate to remove unsaturated uronic acids by a method described by Galand and Marchandise (28). The desulfated disaccharide fraction was precipitated with equal volume of 70% mercapturic acid (pH 5.0) and incubated at room temperature for 10 min. The reaction products, [35S]sulfated glucosamines, were reduced with sodium borohydride as described (41) and analyzed by HPLC on a SAX column as described above.
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Sulfotransferase activities were assayed using various glycosaminoglycans as acceptor substrates as described under “Experimental Procedures.”

| Substrate         | Relative activity |
|-------------------|-------------------|
| Heparan sulfate   | 100               |
| Heparin           | 9.6               |
| Chondroitin sulfate A | 0         |
| Chondroitin sulfate C | 0       |
| Dermatan sulfate  | 0                 |
| Keratan sulfate   | 0                 |
| Hyaluronic acid   | 0                 |

The values indicate the relative rate of the incorporation into various substrates to that into heparan sulfate.

GlcNS,3S) and GlcNS,3S,6S were reduced with sodium [3H]borohydride and used as a standard.

Preparation of 35S-Labeled Heparin and Digestion with Heparin Lyases—35S-Labeled heparin was prepared by incubating 0.3 mg of heparin with [35S]PAPS (2.3 × 10^4 dpm) and 20 μl of immobilized enzyme in a standard reaction mixture. The 35S-labeled heparin was precipitated with ethanol and digested with a mixture of heparin lyases as described above. The digested products were subjected to HPLC on a SAX column as described above. Fractions of 0.24 ml were collected and the radioactivity was determined. A peak fraction at 30.1 min (indicated by a white arrow in Fig. 6) was applied to a Cellulofine G-25-sf column (1 × 28 cm) equilibrated with distilled water to remove LiCl. Radioactive fractions were pooled and concentrated under vacuum.

Quantitative Analysis of the 3-OST-5 Transcripts in Human Tissues by Real-time PCR—For quantification of 3-OST-5 transcripts, we employed the real-time PCR method, as described in detail previously (42). Total RNAs derived from various human tissues were purchased from Clontech and cDNAs were synthesized with the SuperScript First-strand Synthesis System for RT-PCR (Invitrogen). To obtain the control DNA of 3-OST-5, a DNA fragment containing exon I and the 5' strand Synthesis System for RT-PCR (Invitrogen). To obtain the control DNA of 3-OST-5, a DNA fragment containing exon I and the 5’ terminal region of exon II was amplified by PCR using the Marathon Ready cDNA, as a template, and two primers, 5’-CTATCTGACGGCTAGCAGTCAT-3’ and 5’-TCTACTGTCGACGGCCAGGC-3’. Standard curves for the 3-OST-5 and the endogenous control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNAs, were generated by serial dilution of the control DNA of 3-OST-5, and a PCR2.1 vector (Invitrogen) containing the GAPDH gene. The primer sets and the probes for 3-OST-5 were as follows: the forward primer was 5’-GCGAGATAAGG-GAGCTTGC-3’, the reverse primer was 5’-ACCATGCGACCT-CTAATGG-3’, and the probe for 3-OST-5 was 5’-TAGGCTACAC-CCATTGGG-3’ with a minor groove binder (43). PCR products were continuously measured with an ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Foster City, CA). The relative amounts of 3-OST-5 transcripts were normalized to the amount of GAPDH transcript in the same cDNA.

Analysis of Bovine Intestinal Heparan Sulfate—Bovine intestinal heparan sulfate (0.2 mg) was digested with a mixture of heparin lyases as described above. The digested products were reduced with sodium [3H]borohydride and subjected to HPLC on a SAX column as outlined above. 35S-Labeled tetrasulfated disaccharide obtained from 3-OST-5-modified heparin in this study was used with sodium borohydride and used as a standard.

RESULTS

Specificity for Acceptor Substrate—The putative catalytic domain of 3-OST-5 was cloned and expressed, and the sulfotransferase activity was examined as described in “Experimental Procedures.” As expected, 3-OST-5 exhibited sulfotransferase activity toward heparan sulfate and heparin. On the other hand, it exhibited no activity toward chondroitin sulfate A, chondroitin sulfate C, dermatan sulfate, keratan sulfate, and hyaluronic acid. The activity of 3-OST-5 for heparan sulfate is presented as 100%, and the other activities are given as relative values in Table I.

Digestion of 35S-Labeled Heparan Sulfate with Heparin Lyases—35S-Labeled heparan sulfate, which was prepared by incubating heparan sulfate with [35S]PAPS and the recombiant 3-OST-5, was digested with a mixture of heparin lyases and analyzed by HPLC on a SAX column as described under “Experimental Procedures.” When monitored at 232 nm, six known disaccharide units of heparan sulfate, HexA-GlcNAc, HexA-GlcNS, HexA-GlcNS,6S, HexA-GlcNS,6S,2S, HexA-GlcNS,6S,2S, were identified (Fig. 1A). Several radioactive peaks were observed, but no peak was identified at the position of these six units (Fig. 1B). Therefore, the 35S-labeled products were different from these six disaccharides. The digestion products were applied to a gel filtration column of BioGel P-4, and two major 35S radioactive peaks were obtained (Fig. 2). The fractions indicated by a horizontal bar in Fig. 2, named P4-1 and P4-2, were pooled and desalted for further analysis. To confirm the molecular size, these two fractions were analyzed by HPLC on a gel filtration column. The elution profile of standard oligosaccharides prepared from heparin is shown in Fig. 3A. 35S-Labeled substances, P4-1 and P4-2 in Fig. 2, were eluted at the position of the tetrasaccharide and disaccharide, respectively (Fig. 3, B and C). Two fractions were further analyzed by HPLC on a SAX column, and each fraction gave two components. Judging from the elution position, P4-1 and P4-2 corresponded to peaks 1 and 3, and peaks 2 and 4, respectively, which were four major peaks observed in Fig. 1B. To confirm the resistance to enzyme digestion, P4-1 was treated with the heparin lyase mixture again. No change was observed in the appearance of peak 1 and peak 3 during HPLC analysis on a SAX column. Moreover, this fraction is also resistant to nitrous acid degradation. The P4-1 was not characterized further, since these tetrasaccharides

FIG. 1. HPLC analysis on a SAX column of 35S-labeled products derived from heparan sulfate. 35S-Labeled heparan sulfate produced by the sulfotransferase reaction of 3-OST-5 was digested with a mixture of heparin lyases as described under “Experimental Procedures.” The digested product was analyzed by HPLC on a SAX column. The conditions for HPLC were as described under “Experimental Procedures.” The absorbance was monitored at 232 nm (panel A). Fractions of 0.22 ml were collected, and radioactivity was determined by liquid scintillation counting (panel B). The arrows in panel A indicate the elution positions of HexA-GlcNAc (ΔDi-NS), HexA-GlcNS,6S,2S, and HexA-GlcNS,6S,2S. The radioactive peaks were obtained (Fig. 2). The fractions indicated by a horizontal bar in Fig. 2, named P4-1 and P4-2, were pooled and desalted for further analysis. To confirm the molecular size, these two fractions were analyzed by HPLC on a gel filtration column. The elution profile of standard oligosaccharides prepared from heparin is shown in Fig. 3A. 35S-Labeled substances, P4-1 and P4-2 in Fig. 2, were eluted at the position of the tetrasaccharide and disaccharide, respectively (Fig. 3, B and C). Two fractions were further analyzed by HPLC on a SAX column, and each fraction gave two components. Judging from the elution position, P4-1 and P4-2 corresponded to peaks 1 and 3, and peaks 2 and 4, respectively, which were four major peaks observed in Fig. 1B. To confirm the resistance to enzyme digestion, P4-1 was treated with the heparin lyase mixture again. No change was observed in the appearance of peak 1 and peak 3 during HPLC analysis on a SAX column. Moreover, this fraction is also resistant to nitrous acid degradation. The P4-1 was not characterized further, since these tetrasaccharides
activity was determined. The fractions indicated by horizontal bars were digested with 2-sulfatase as described under “Experimental Procedures.” Fractions of 2 ml were collected, and radioactivity was determined. The fractions indicated by horizontal bars, named P4-1 and P4-2, were pooled.

Encountered some difficulty in further identification using enzymes (see “Discussion”).

Structural Analysis of $^{35}$S-Labeled Disaccharides from Heparan Sulfate—The $^{35}$S-labeled disaccharide fraction (P4-2) was digested with 2-sulfatase as described under “Experimental Procedures,” and analyzed by HPLC on a SAX column (Fig. 4A). With the enzyme digestion (Fig. 4B), only one component, peak 2 in panel A, shifted its retention time to an earlier position. The result indicates that peak 2 has a $\Delta$HexA(2S) residue, but not peak 1. To confirm the location of the sulfate group of glucosamine residues, P4-2 was treated with mercuric acetate to remove unsaturated uronic acids. The reaction products were reduced with sodium borohydride and analyzed by HPLC on a SAX column (Fig. 5A). By comparison with $^3$H-labeled standards (Fig. 5B), peak a and peak b were identified as reduced forms of GlcN(NS,3S) and GlcN(NS,3S,6S). Judging from peak area, peak a and peak b originated from peak 2 and peak 1 in Fig. 4A, respectively. From these data, peak 1 and peak 2 in Fig. 4A were determined as $\Delta$HexA-GlcN(NS,3S,6S) and $\Delta$HexA(2S)-GlcN(NS,3S), respectively. Fig. 9, A and B show schematic drawings of the analysis described above.

Highly Sulfated Structure of $^{35}$S-Labeled Heparin—$^{35}$S-Labeled heparin was prepared by incubating heparin with $[^{35}$S]PAPS and the recombinant 3-OST-5 and digested with a mixture of heparin lyases as described under “Experimental Procedures.” When the digested products were separated by HPLC on a SAX column, major radioactivity was detected at the retention time of 30.1 min, later than the positions of trisulfated disaccharides (Fig. 6). The fraction indicated by a white arrow in Fig. 6 was pooled and desalted for further analysis. The $^{35}$S-labeled product was confirmed to be a disaccharide isolated from 3-OST-5-modified heparin was determined as $\Delta$HexA-GlcN(NS,3S,6S) as described above. Therefore, $\Delta$HexA(2S)-GlcN(NS,3S,6S) was expected. To confirm the location of the sulfate group of glucosamine residue, $^{35}$S-labeled disaccharide was treated with mercuric acetate, reduced with sodium borohydride, and analyzed by HPLC on a SAX column (Fig. 5A). The position of the $^{35}$S radioactive peak corresponded to that of $^3$H-labeled GlcN (NS,3S,6S) standard (Fig. 5B). From these data, the $^{35}$S-labeled disaccharide from 3-OST-5-modified heparin was determined...
Quantitative Analysis of the 3-OST-5 Transcripts in Human Tissues—We determined the tissue distribution and expression levels of the 3-OST-5 transcripts by a real-time PCR method. The expression levels of 3-OST-5 in various tissues were shown as a relative amount compared with the GAPDH transcripts (Fig. 10). The transcripts were highly expressed in fetal brain, followed by adult brain and spinal cord. Cerebellum, colon and skeletal muscle expressed the 3-OST-5 transcripts at a relatively low level. The expression levels in the remaining tissues were very low or undetectable.

Identification of Tetrasulfated Disaccharide in Bovine Intestinal Heparan Sulfate—To confirm the presence of the natural tetrasulfated disaccharide unit in heparan sulfate, we chose bovine intestinal heparan sulfate as a natural source because of the availability of highly purified material. The bovine intestinal heparan sulfate was digested with a mixture of heparin lyases as described under “Experimental Procedures.” The digested products were reduced with sodium borohydride and analyzed by HPLC on a SAX column (panel A). Panel B shows analysis of sulfated glucosamine standards, GlcN(NS,3S) and GlcN(NS,3S,6S), reduced with sodium $[^3H]$borohydride. A letter R attached to the name means reduced form.
elution position is shown in Fig. 11 as ΔDi-tetraSR. Weak but apparent radioactivity was detected at this position (inset of Fig. 11). To confirm the molecular size, the peak fraction (indicated by a white arrow) was analyzed by the HPLC on a gel filtration column. The 3H-labeled substance was eluted at the position of the tetrasulfated disaccharide standard (data not shown).

**DISCUSSION**

The cloning and characterization of a sixth member of the human 3-OST family, named 3-OST-5, was recently reported. Xia et al. (35) employed low pH nitrous acid degradation to analyze the reaction products and identified three 3-O-sulfated disaccharides, GlcA-aMan(3S,6S), IdoA(2S)-aMan(3S), and IdoA(2S)-aMan(3S,6S). In the case of 3-O-sulfated glucosamine, both N-sulfated and N-unsulfated residues are susceptible to low pH nitrous acid degradation, therefore the N-substituents of the glucosamine residue were unidentified (31). They also confirmed that the 3-OST-5-modified heparan sulfate binds to AT III and gD. In addition, transfection of the plasmid expressing 3-OST-5 makes CHO cells susceptible to HSV-1. From these results, they concluded that 3-OST-5 has the activities of both 3-OST-1 and 3-OST-3. The reaction specificity of 3-OST-1 and 3-OST-3 is the formation of GlcA-GlcN(6S) and Ido(2S)-GlcN(6S), respectively (31, 44).

In the present study, we employed enzyme digestion to analyze the reaction products. 35S-Labeled heparan sulfate, which was prepared by incubating heparan sulfate with [35S]PAPS and recombinant 3-OST-5, was digested with a mixture of heparin lyases. Two major 35S-labeled disaccharides obtained from the digests were determined as ΔHexA-GlcN(NS,3S,6S) and ΔHexA(2S)-GlcN(3S,6S), respectively (31, 44).

It has been believed that the glucosaminidic linkage adjacent to a disaccharide unit containing a 3-O-sulfated glucosamine residue is resistant to heparin lyases (45, 46). To date, unsaturated disaccharides containing a 3-O-sulfated glucosamine...
3-OH was reduced with sodium [3H]borohydride and separated by HPLC on a SAX column. The conditions for HPLC were as described under “Experimental Procedures.” Fractions of 0.24 ml were collected, and radioactivity was determined. Six known disaccharides were identified in comparison with the disaccharide standards reduced with sodium borohydride. Abbreviations of disaccharides are shown in the legend of Fig. 1. A letter R attached to the name means reduced form. [35S]-Labeled ΔHexA(2S)-GlcN(NS,3S,6S) obtained from 3-OST-5-modified heparin as described in the present study was reduced with sodium borohydride and used as a standard. Its elution position is indicated in the legend of Fig. 1. A letter R attached to the name means reduced form. Abbreviations of disaccharides are shown in the legend of Fig. 1. A letter R attached to the name means reduced form.

Although 3-OST-5 is the only enzyme known to catalyze the formation of the tetrasulfated disaccharide unit at this time, there is no evidence that the novel structure detected in this study was synthesized by the 3-OST-5 in bovine intestine. The generation of transgenic and gene knockout animal models could provide not only the answer to this question but also important information about the biological role of 3-OST-5 or its reaction products including tetrasulfated disaccharide.

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