Crystal Structure of the Sulfo transferase Domain of Human Heparan Sulfate N-Deacylase/N-Sulfotransferase 1*

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Heparan sulfate N-deacetylase/N-sulfotransferase (HSNST) catalyzes the first and obligatory step in the biosynthesis of heparan sulfates and heparin. The crystal structure of the sulfotransferase domain (NST1) of human HSNST-1 has been determined at 2.3-Å resolution in a binary complex with 3'-phosphoadenosine 5'-phosphate (PAP). NST1 is approximately spherical with an open cleft, and consists of a single αβ fold with a central five-stranded parallel β-sheet and a three-stranded anti-parallel β-sheet bearing an interstrand disulfide bond. The structural regions α1, α6, β1, β7, 5'-phosphosulfate binding loop (between β1 and α1), and a random coil (between β8 and α13) constitute the PAP binding site of NST1. The α6 and random coil (between β2 and α2), which form an open cleft near the 5'-phosphate of the PAP molecule, may provide interactions for substrate binding. The conserved residue Lys-614 is in position to form a hydrogen bond with the bridge oxygen of the 5'-phosphate.

Heparan sulfate chains are ubiquitous as proteoglycans on cell surfaces and in the extracellular matrix. They have been increasingly implicated in various biological processes including cell growth, cell differentiation, coagulation, and viral and bacterial infections (1, 2). Reduced biosynthesis of heparan sulfates may affect cell growth, cell differentiation, blood coagulation, and viral infections (1, 2). Reduced biosynthesis of heparan sulfates and heparin is increasingly implicated in various biological processes including cell growth, cell differentiation, blood coagulation, and viral infections (1, 2). Reduced biosynthesis of heparan sulfates may affect cell growth, cell differentiation, blood coagulation, and viral infections (1, 2).

EXPERIMENTAL PROCEDURES

Protein Expression, Purification, Crystallization, and Enzyme Assay—Selenomethionyl NST1, using a pGEX-4T3-NST1 plasmid, was expressed in the methionine auxotrophic Escherichia coli strain B834 (DE3) with a defined minimal essential medium (without methionine) containing 50 mg of selenomethionine per liter of culture. The NST1 was then purified, and crystals (P2₁2₁2₀ or P 2₁) were grown under the same conditions as described previously (14). Heparan sulfate sulfotransferase activity of NST1 was also measured according to the previously described procedure (12).

Crystallographic Data Collection and Processing—Two MAD data sets of selenomethionyl NST1 were collected at −180 °C from two separate single crystals (both P2₂2₂) on a MAR detector at beamline X9B of the NSLS. Brookhaven National Laboratory. Three wavelengths were selected from the fluorescence spectra: f₁ (0.97163 Å; remote), f₂ (0.97907 Å; peak), and f₃ (0.97940 Å; edge) (Table I). Native data of an NST1 crystal (P2₁) were collected at −180 °C on an R-axis IV with an RU300 rotating anode generator.

Structure Determination and Refinement—All data were processed using SCALEPACK and DENOZO (14). Because of heavy ice rings in data set 1 and data set 2 being weak, F(±)s were calculated with CCP4 (15) using data between 20 and 4 Å of data set 1. Positions for four of the six selenium atoms were determined using SHELX96 (16). Data set 1 was

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The abbreviations used are: PAPS, 3'-phosphoadenosine 5'-phosphosulfate; PAP, 3'-phosphoadenosine 5'-phosphate; PSB-loop, 5'-phosphosulfate binding loop; 5'PSB, 5'-phosphosulfate binding motif; 3'PB, 3'-phosphate binding motif; NST1, the sulfotransferase domain of heparan sulfate N-deacetylase/N-sulfotransferase; EST, estrogen sulfotransferase.

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reprocessed to eliminate all reflection near the ice rings between 3.95 and 3.1 Å. Subsequently, the reprocessed data set 1 was merged with data set 2 to obtain a complete data set to 2.85-Å resolution. SHARP (17) was then used for refinement of the selenium sites. Solvent flattening and histogram matching were carried out using DM and Solom from CCP4 (15). In the model building process using O (18), SigmaA maps were generated by combining the phases from polyalanine fragments with the MAD phases (15). After multiple cycles of refinement and histogram matching were carried out using DM and Solo-

The overall structure of NST1 is roughly spherical with an open cleft (Fig. 1A). This structure is composed of a five-stranded parallel β-sheet (β1, β2, β3, β4, and β5) with α helices on both sides of the β-sheet (Fig. 1B). This fold is similar to EST (the 1.9-Å r.m.s. deviation for 97 Cos in β1, β3, β4, β5, α1, α6, α11, α12, and α13) as well as to the nucleotide binding motif observed in nucleotide kinases (20). The loop between β1 and α1 adopts the same PSB-loop configuration as the 5'-phosphate binding site of PAPS. A cavity formed between the PSB-loop, and α6 defines the PAP binding site. Three β strands (β6, β7, and β8) near the C terminus form an anti-parallel β-sheet with a single disulfide bond between β7 and β8. An open cleft that runs perpendicular to the PAP binding cavity is large enough to contain a hexaaspartic acid chain. The α6 and random coil between β2 and α2 constitute the cleft near the 5'-phosphate of PAP and thus may constitute part of the substrate binding site.

The secondary structural elements that comprise the PAP binding site in NST1 and residues forming specific interactions to the PAP molecule are depicted (Fig. 2), respectively. The PSB-loop (residues 612–617) and α1 of NST1 constitute the 5'-PSB motif and provide the major binding sites for the 5'-phosphate of the PAP molecule. Backbone amide nitrogens from PSB-loop residues 614–618 are all within hydrogen bonding distance of the 5'-phosphate. The side-chain Nε of Lys-614 and the Oγ of both Thr-617 and Thr-618 are also hydrogen-bonded to the 5'-phosphate. α6 and β4 are the key elements of the 3'PB motif, and the Oγ of Ser-712 from this helix forms a hydrogen bond to the 3'-phosphate of the PAP molecule. The PAP molecule in NST1 is bound in the same orientation (relative to the PSB-loop) as seen in EST. The PAP binding site, found in the EST structure determined previously, is conserved. The r.m.s. deviation (with EST) for 47 Cos in α1, α6, β1, β4, and PSB-loop is 1.16 Å.

The anti-parallel β-sheet (β6, β7, and β8) and the following random coil provide the remaining interactions for the PAP binding site (Fig. 2). These interactions reveal diversity in the binding site of NST1. The side-chains of Lys-835 and Tyr-837 from this random coil are within hydrogen bonding distance to two oxygen atoms of the 5'-phosphate and the oxygen atom of the 3'-phosphate, respectively. Besides these side-chain interactions, the backbone nitrogens of Gly-834 and Arg-835 are also within hydrogen bonding distance of a 3'-phosphate oxygen of the PAP molecule. The adenine ring from the PAP molecule is in position to form a parallel ring stacking interaction with Phe-816 of β7. Moreover, the backbone oxygen of Trp-817 is within hydrogen bonding distance to the N of the adenine. The interactions of these residues with the PAP molecule are unique features in NST1 that are not present in the crystal structure of the EST-PAP complex (8).

Lys-614 of NST1 is known to be conserved in other heparan sulfate sulfto transferases as well as in all cytosolic sulfotransf erase (9, 12). Although this residue plays a critical role in NST1 activity (12), the structural basis of its role in catalysis has remained unresolved. The crystal structure of the EST-

TABLE I

| Data collection and refinement statistics | Data set | f1 (set 1) | f2 (set 1) | f3 (set 1) | f1 (set 2) | f2 (set 2) | f3 (set 2) | native |
|----------------------------------------|----------|-----------|-----------|-----------|-----------|-----------|-----------|--------|
| Space group                            | P21212   | P21212    | P21212    | P21212    | P21212    | P21212    | P21212    | P21    |
| Unit cell parameters (Å)               | a = 89.13| a = 89.50 | a = 89.44 | a = 89.24 | a = 88.73 | a = 88.46 | a = 45.42 |
|                                        | b = 55.36| b = 55.60 | b = 55.55 | b = 55.42 | b = 55.25 | b = 55.15 | b = 54.50 |
|                                        | c = 66.41| c = 67.01 | c = 66.92 | c = 66.68 | c = 66.44 | c = 66.36 | c = 68.94 |
| No. of crystals                         | 1        | 1         | 1         | 1         | 1         | 1         | 1         |
| Resolution (Å)                         | 2.85     | 2.85      | 2.85      | 3.2       | 3.2       | 3.2       | 2.3       |
| % Completeness (total)                 | 79.5     | 83.7      | 80.5      | 100       | 100       | 99.9      | 93.7      |
| % Completeness (last shell)            | 99.6     | 90.2      | 85.7      | 100       | 100       | 100       | 80        |
| No. of unique reflections              | 6439     | 6897      | 6613      | 5800      | 5731      | 5705      | 13992     |
| Refinement statistics                  | native   |           |           |           |           |           |           |
| Resolution (Å)                         | 50–2.3   |           |           |           |           |           |           |
| Rfree/Rfree (%)                       | 21.0/25.7|           |           |           |           |           |           |
| No. of waters                          | 73       |           |           |           |           |           |           |
| r.m.s. deviations from ideality        |           |           |           |           |           |           |           |
| Bond lengths (Å)                       | 0.007    |           |           |           |           |           |           |
| Bond angles (deg)                      | 1.3      |           |           |           |           |           |           |
| Dihedral angles (deg)                  | 25.0     |           |           |           |           |           |           |
| Improper angles (deg)                  | 0.65     |           |           |           |           |           |           |

a Rsym = ΣIi − <I>/ΣIi, where Ii is the intensity of the ith observation and <I> is the mean intensity of the reflection.

b R = Σ|Fobs| - |Fcal| / Σ|Fobs|, where Rsym is calculated using 95% of the reflections in refinement and Rfree is calculated using the remaining 5%.
is also coordinated to Lys-48 (Nζ) in EST and is implicated as the bridge oxygen of the leaving phosphate group of PAP (21). Moreover, the mutation of Lys-614 to Arg gives a variant with a significant level of NST1 activity (63 ± 6.0 and 9.4 ± 2.4 nmol of sulfate/min/mg of protein in the wild-type and K614R mutant, respectively), whereas the K614A mutation abolishes activity completely (12). These structural and mutational data suggest that Lys-614 may act as a possible proton donor in catalysis, similar to Lys-48 in EST (21). Lys-833 of NST1 is also coordinated with the bridge oxygen (Fig. 3). Lys-833 is, in fact, conserved not only in Caenorhabditis elegans HSNST but also in human heparan sulfate 3-O-sulfotransferase (see the sequence alignments in Shworak et al. (22)). Thus, Lys-833 and its counterparts may play a significant role in catalysis.

In sharp contrast to the hydrophobic pocket of estrogen binding site in EST, the putative substrate binding site of NST1 appears to be a large open cleft with a hydrophilic surface, with a random coil (residues 640–647, approximately 12 Å in length) and a6 forming the center of the cleft near the 5’-phosphate of the PAP molecule. This amphipathic random coil positions negatively charged side-chains (Glu-641, Glu-642, Gln-644, and Asn-647) toward the center, whereas the hydrophobic side-chains (Ile-643, Phe-645, and Phe-656) are buried in the hydrophobic core of NST1. The side-chains of residues (Trp-713, His-716, Gln-717, and His-720) in a6 constitute the opposing face of the cleft. The center of this cleft (approximate dimensions: 12 Å in length, 8 Å in width, and 8 Å in depth) is large enough to accommodate a trisaccharide unit of polysaccharide chain. Further studies, such as the determination of the complex structure of NST1 complexed with polysaccharide, are needed to conclude whether this center portion of the cleft is, in fact, the substrate binding site.

CONCLUSION

The striking similarities between the PAP binding orientation in NST1 and EST provide structural evidence that the Golgi membrane and cytosolic enzymes belong to the same family of enzymes. The similar topology and function of Lys-614 to Lys-48 of EST suggest a common reaction mechanism in all sulfotransferases. Lys-833 may be an additional catalytic residue not present in the cytosolic enzymes. The NST1 structure provides an excellent model for investigating the substrate specificity of heparan sulfate sulfotransferases so that we may better understand sulfation at specific positions of glucuronic acid-N-acetylgalactosamine.

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FIG. 3. Superimposition of the 5′-phosphate binding site of NST1 with the active site of the EST-PAP-vanadate complex. NST1 and its PAP are shown using carbons in gray, oxygens in red, phosphorus atom of PAP in yellow, and nitrogens in blue. Both the EST and its PAP are in green. Dotted lines indicate possible transition state coordinations, as judged from their hydrogen bonding distances (red for NST1, yellow for EST). The vanadate ion is shown at the center of the active site (vanadium atom is cyan, oxygens are red). The red circle shows the position of the apical water molecule in the EST-PAP-vanadate complex (21). This figure was created in SETOR (23).