Electrophoresis based sulfotransferase assay using a variety of glycan & peptide substrates

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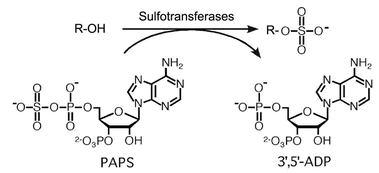
ABSTRACT

Sulfotransferases are a large group of enzymes that are involved in various biological functions. They regulate the biological activity or availability of a wide spectrum of substrates through sulfation with the activated sulfur donor 3'-phosphoadenosine-5'-phosphosulfate (PAPS). Two major categories of sulfotransferases are found in mammals based on their subcellular location. Cytoplasmic sulfotransferases are mainly involved in modifying small molecules, such as steroids, neurotransmitters, and xenobiotics. Golgi resident sulfotransferases, on the other hand, are involved in the modification of glycans and proteins found on the cell membrane and extracellular matrix. Assaying these sulfotransferases can be a challenge. Traditionally, sulfotransferase assays rely on chromatography steps, such as HPLC and TLC, to separate products from substrates. Here, we describe a new sulfotransferase assay method based on electrophoresis. The acceptor substrates can be as small as a disaccharide and as large as a proteoglycan. The donor substrate is spiked with radioactive ^{35}S PAPS. After sulfation, the product and substrate are separated with polyacrylamide gel electrophoresis and visualized by radiograms. As examples, we present several sulfotransferase assays using oligosaccharides, polysaccharides, peptides, and recombinant proteoglycans as acceptor substrates.

METHODS

A. Sulfotransferase reaction



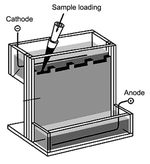
B. Assay procedure

1. Add substrates to reaction buffer

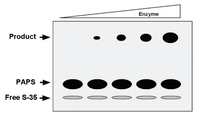
2. Spike with 10^6 CPM of ^{35}S PAPS

3. Add enzyme and incubate at 37°C for 20 minutes

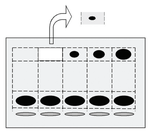
4. Electrophoresis with 8% SDS polyacrylamide gel



5. Dry the gel and expose to X-ray film



6. Cut the hot spots and count with a scintillation counter



7. Calculations

$$\text{Activity} = S \times \frac{C_i}{C_t} \times \frac{1}{t}$$

S = amount of donor substrate
C_i = incorporated counts
C_t = total applied counts
t = reaction time (min)

Specific activity is the linear slope of a plot of activity versus enzyme amount

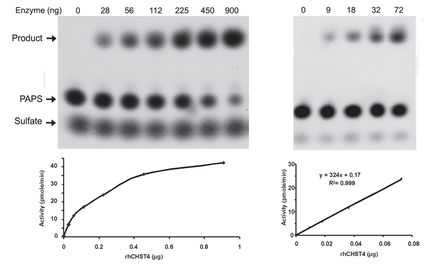
Electrophoresis buffer: 40 nM Tris (pH 8.0), 40 mM acetic acid, 1 mM EDTA

Reaction buffer: 25 mM MES (pH 7.0), 0.5% (w/v) Triton X-100, 2.5 mM $MgCl_2$, 2.5 mM $MnCl_2$ 1.25 mM $CaCl_2$, 0.75 mg/mL BSA

Sulfotransferases: purified from conditioned media of CHO cells expressing human or mouse enzymes

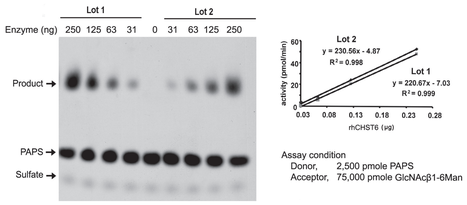
Figure 1: Sulfotransferase assay with oligosaccharides

A. rhCHST4 assay with GlcNAcβ1-6Man



CHST4, or high endothelial cell N-acetylglucosamine 6-O-sulfotransferase (HEC-GlcNAc6ST), or L-selectin ligand sulfotransferases (LSST), transfers sulfate to position 6 of non-reducing N-acetylglucosamine (GlcNAc) residues within mucin-associated glycans that ultimately serve as L-selectin ligands. It has a catalytic preference for core 2-branched mucin-type O-glycans, but also has activity toward core 3 type of O-glycan. The left radiogram was for the enzymatic curve and the right radiogram was for the activity measurement. The specific activity for rhCHST4 under the specified condition was around 300 pmole/min/µg.

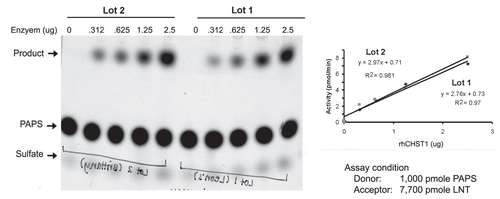
B. rhCHST6 assay with GlcNAcβ1-6Man



Assay condition
Donor, 2,500 pmole PAPS
Acceptor, 75,000 pmole GlcNAcβ1-6Man

CHST6, or corneal N-acetylglucosamine-6-O- sulfotransferases, transfers sulfate to position 6 of non-reducing N-acetylglucosamine (GlcNAc) residues of keratan. Keratan sulfate plays a central role in maintaining corneal transparency. It acts on the non-reducing terminal GlcNAc of short and long carbohydrate substrates that have poly-N-acetyllactosamine structures. Defects in CHST6 are the cause of macular corneal dystrophy (MCD). Under the specified condition, the two lots of rhCHST6 showed specific activity around 220 pmole/min/µg.

C. rhCHST1 assay with Lacto-Neo-tetraose (LNT)

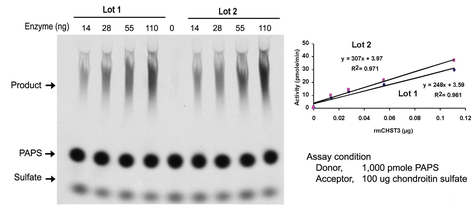


Assay condition
Donor: 1,000 pmole PAPS
Acceptor: 7,700 pmole LNT

CHST1, or keratan sulfate Gal-6 sulfotransferase, transfers sulfate to position 6 of galactose residues of keratan. It has a preference for sulfating keratan sulfate, but it also transfers sulfate to the unsulfated polymer. It participates in biosynthesis of selectin ligands that play a central role in lymphocyte homing at sites of inflammation. In this assay, we used unsulfated lacto-neo-tetraose as acceptor substrate. Under the specified condition, the two lots of rhCHST1 showed specific activity around 3 pmole/min/µg.

Figure 2: Sulfotransferase assay with glycosaminoglycans

rmCHST3 with chondroitin sulfates

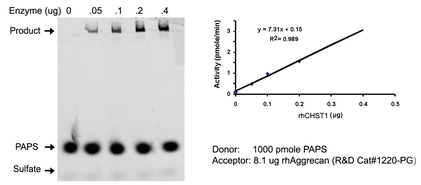


Assay condition
Donor, 1,000 pmole PAPS
Acceptor, 100 ug chondroitin sulfate

CHST3, also known as chondroitin 6-sulfotransferase, catalyzes the transfer of sulfate to position 6 of the N-acetylgalactosamine (GalNAc) residue of chondroitin. Chondroitin sulfate constitutes the predominant proteoglycan present in cartilage and is distributed on the surfaces of many cells and extracellular matrices. Defects in CHST3 are the cause of spondyloepiphyseal dysplasia Omani type (SED Omani type), an autosomal recessive disorder characterized by normal length at birth but severely reduced adult height (110-130 cm). Under the specified condition, the two lots of rmCHST3 showed specific activity around 270 pmole/min/µg.

Figure 3: Sulfotransferase assay with proteoglycans

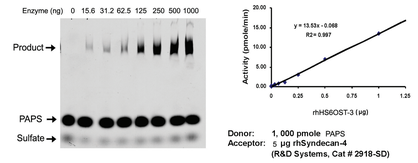
A. rhCHST1 assay with recombinant aggrecan



Donor: 1000 pmole PAPS
Acceptor: 8.1 ug rhAggrecan (R&D Cat#1220-PG)

rhCHST1 was also assayed with recombinant aggrecan as the acceptor substrate. Aggrecan is a typical large keratan sulfate/chondroitin sulfate proteoglycan in cartilagenous tissue. Native aggrecan consists of two globular structural domains at the N-terminal end and one globular domain at the C-terminal end, separated by a large domain heavily modified with glycosaminoglycans. The recombinant aggrecan consists of two N-terminal globular domains and contains about 30 kDa keratan sulfate as indicated by deglycosylation study. Under the specified condition, the specific activity of rhCHST1 was measured to be about 8 pmole/min/µg.

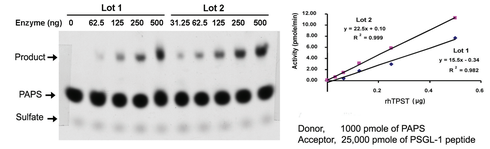
B. rmHS6ST-3 assay with recombinant Syndecan-4



Donor: 1,000 pmole PAPS
Acceptor: 5 µg rhSyndecan-4
(R&D Systems, Cat # 2918-SD)

Heparan sulfate 6-O sulfotransferase 3 (HS6ST-3) transfers sulfate to the 6-O position of the GlcNAc residues on heparan sulfate (HS). Sulfation is essential for the biological functions of HS. Other sulfation on HS includes 2-O sulfation on the hexauronic acid residues and 3-O and N- sulfation on the GlcNAc residues. Syndecans are type 1 transmembrane proteoglycans that constitute the major physiological form of HS on the cell surface. HS side chains serve as binding sites for growth factors, cytokines, and extracellular matrix proteins. HS contributed about 15 kDa to the molecular weight of the recombinant Syndecan-4. Under the specified condition, rhHS6OST-3 showed specific activity of 14 pmole/min/µg.

Figure 4: Tyrosine protein sulfotransferase assay with PSGL-1 peptide



Donor, 1000 pmole of PAPS
Acceptor, 25,000 pmole of PSGL-1 peptide

Sulfation on the P-selectin glycoprotein ligand-1 (PSGL-1) on myeloid cells and stimulated T lymphocytes plays a critical role in the tethering of these cells to activated platelets or endothelia expressing P-selectin. Two tyrosine protein sulfotransferases (TPST) have been found in the human genome. Here we assayed recombinant human TPST1 with the substrate PSGL-1 peptide (Q42ATEYEYLDYDFLPET57). The specific activity for lot 1 was significantly lower than that of lot 2, which could be due to long time span (two years apart) between the productions of the two lots.

SUMMARY

- Gel electrophoresis can be applied to assaying sulfotransferases with a wide spectrum of substrates
- Glycoproteins (such as proteoglycans) in addition to glycans can be excellent acceptors for sulfotransferases
- Sulfotransferases can be used as enzymatic tools to introduce isotope-labeled sulfate into glycans and proteins

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