Detection of Specific Glycosaminoglycans and Glycan Epitopes by *in vitro* Sulfation using Recombinant Sulfotransferases

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ABSTRACT

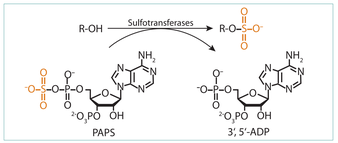
Sulfated glycans play a critical role during the development, differentiation and growth of various organisms. The most well-studied sulfated molecules are sulfated glycosaminoglycans (GAGs). Sulfation at any particular site on a glycan is usually unsaturated *in vivo*. Therefore, it is possible to introduce the ^{35}S radioisotope to glycans using specific recombinant sulfotransferases *in vitro*, allowing detection of minute quantities of these glycans. As examples, we tested for contaminant GAGs in commercial heparin, heparan sulfate, and chondroitin samples. The identities of the contaminating GAGs were further confirmed by digestion using GAG-specific lyases. This strategy can also be used to detect oversulfated chondroitin sulfate following a simple desulfation step. In addition, this method of *in vitro* sulfation by sulfotransferases allowed us to map glycan epitopes in biological samples. This was illustrated using mouse embryo and rat organ tissue sections labeled with different sulfotransferases, including CHST3, CHST15, HS3ST1, CHST4, and CHST10.

INTRODUCTION

- The most well-known sulfated molecules are glycosaminoglycans (GAGs). GAGs are linear amino polysaccharides that are generally found in the extracellular matrix and on cell membranes. Sulfated GAGs include chondroitin sulfate, heparan sulfate, heparin, dermatan sulfate, and keratan sulfate.
- GAGs are known to be involved in numerous diseases, such as mucopolysaccharidoses, macular corneal dystrophy, osteoarthritis, hereditary multiple exostoses, and herpes simplex infection. Heparin, on the other hand, is used as a treatment for blood coagulation.
- Sulfation is also prevalent on various O-glycans, N-glycans and glycolipids, such as sialyl 6-sulfo Lewis X and human natural killer-1 (HNK-1) carbohydrate.
- Glycan sulfation in humans is carried out by more than 30 sulfotransferases. These enzymes use 3'-phosphoadenosine-5'-phosphosulfate (PAPS) as the sulfur donor. Sulfation *in vivo* is usually not saturated, leaving room for *in vitro* enzymatic sulfation.
- Due to similar chemical and physical characteristics, GAGs are difficult to detect and distinguish from one another. A recent incident with contaminated pharmaceutical heparin conveys the urgency for a sensitive and convenient method to detect different GAGs.

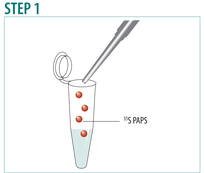
EXPERIMENTAL PROCEDURES

A. SULFOTRANSFERASE REACTION



B. ASSAY PROCEDURES FOR GAG IDENTIFICATION

STEP 1

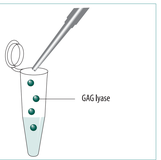


Add ^{35}S PAPS to the GAG sample in sulfotransferase buffer.

STEP 2

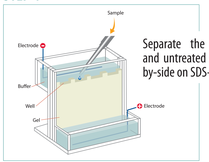
Add a specific sulfotransferase and incubate to allow maximum radioisotope incorporation.

STEP 3



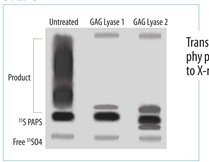
Digest the labeled GAG to completion with a specific GAG lyase.

STEP 4



Separate the lyase-treated and untreated samples side-by-side on SDS-PAGE.

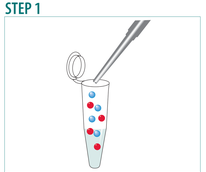
STEP 5



Transfer to chromatography paper, dry, and expose to X-ray film.

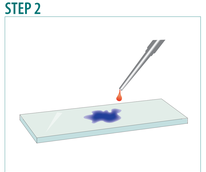
C. ASSAY PROCEDURES FOR ORGAN & TISSUE SECTION LABELING

STEP 1



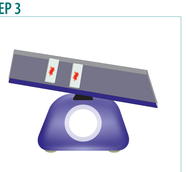
Prepare the labeling mix containing ^{35}S PAPS and a desired sulfotransferase.

STEP 2



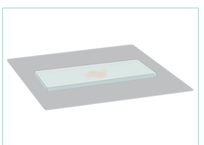
On a flat surface, cover each tissue section with a sufficient amount of the labeling mix.

STEP 3



Gently rock the slides for 40 minutes to evenly expose the tissue section. Wash the slides thoroughly with PBS and then quickly with water.

STEP 4



Air dry the slides and expose to X-ray film.

Electrophoresis buffer: 40 mM Tris (pH 8.0), 40 mM acetic acid, 1 mM EDTA
Sulfotransferase 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 the human or mouse enzymes

FIGURE 1

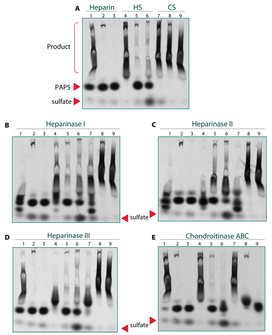


FIGURE 1 Detection of contaminating GAGs in commercial preparations. (A) 10 µg each of commercial heparin (lane 1, 2, 3), heparan sulfate (lane 4, 5, 6) and chondroitin sulfate (lane 7, 8, 9) were sulfated using 0.5 µg of HS6ST1 (lane 1, 4, 7), CHST3 (lane 2, 5, 8), or CHST15 (lane 3, 6, 9). The same set of reactions in (A) was further digested with heparinase I (B), heparinase II (C), heparinase III (D) and chondroitinase ABC (E) to completion.

FIGURE 2

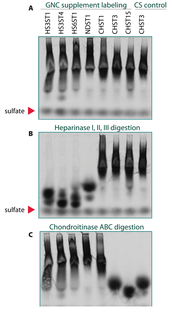
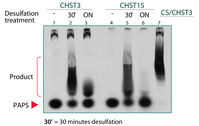


FIGURE 2 Detection of contaminating GAGs in a chondroitin sulfate supplement. (A) A Resuspended GNC supplement pill was labeled using various sulfotransferases. In each reaction, 100 µg of purported chondroitin sulfate from the GNC pill was labeled with 0.5 µg of the indicated sulfotransferase. The last lane was a positive control with the same amount of chondroitin sulfate from Sigma Aldrich labeled with CHST3. (B) The labeled samples were digested with a mixture of heparinase I, II and III. (C) The labeled samples were digested with Chondroitinase ABC.

FIGURE 3



30' = 30 minutes desulfation
ON = overnight desulfation
CS = chondroitin sulfate

FIGURE 3 Detection of oversulfated chondroitin sulfate. Oversulfated chondroitin sulfate could not be labeled by either CHST3 or CHST15 (lane 1 and 4). Following acid desulfation for 30 minutes (30'), the polysaccharides were labeled efficiently by both enzymes (lane 2 and 5). Overnight (ON) desulfation caused the labeling to be less efficient due to depolymerization of the polysaccharide backbone (lane 3 and 6). Lane 7 contained chondroitin sulfate (CS) labeled by CHST3. Each reaction contained 5 µg of chondroitin sulfate and 1 µg of sulfotransferase.

FIGURE 4

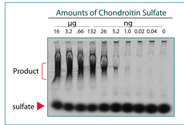


FIGURE 4 Sensitivity of *in vitro* enzymatic sulfation of chondroitin sulfate. Decreasing amounts of chondroitin sulfate from 16 µg to 0.04 ng were labeled with CHST15. The last lane contained no acceptor chondroitin sulfate. For maximum sensitivity, the reactions was carried out overnight.

FIGURE 5

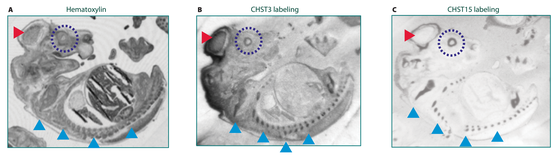


FIGURE 5 Mouse embryo histological sections labeled with sulfotransferases. Each consecutive 5 µm section was stained with hematoxylin (A), CHST3 (B), or CHST15 (C).

FIGURE 6

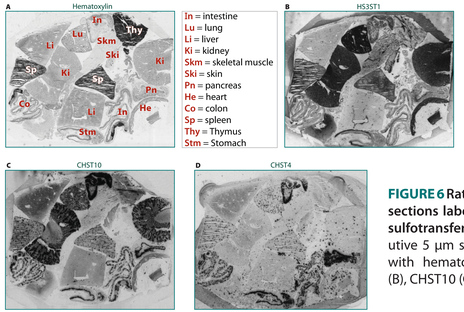


FIGURE 6 Rat organ histological sections labeled with different sulfotransferases. Each consecutive 5 µm section was stained with hematoxylin (A), HS3ST1 (B), CHST10 (C), or CHST4 (D).

CONCLUSION

1. This method can be used for detecting minute amounts of particular GAGs in a mixture.
2. This method can be used for mapping specific unsulfated precursor glycans in tissue sections.
3. The specificity is dependent upon the use of specific sulfotransferases and digestion enzymes. The combination of the two different classes of enzymes provides a much higher level of confidence in the identity of the GAG.
4. This method is simple to perform. Except for oversulfated samples, no pretreatment of the samples is required. No laborious separation steps and no expensive instrumentation is required.