SHORT COMMUNICATION

Structure of heparan sulfate from rat kidney and liver

Aiko Kamada, Masahiro Kawamura, Ken-ichi Nakamichi
and Tetsuya Sakaki

*Department of Biochemistry, Osaka Dental University,
5-31 Otemae 1-chome, Chuo-ku, Osaka 540, Japan*

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Introduction

Heparan sulfate (HS) is covalently attached to protein (HSPG) and widely distributed in mammalian tissues and cultured cells. Recent studies have shown that HS is associated with the outer surface of cells¹⁾ and is a constituent of basement membranes²⁾ and of the extracellular matrix produced by cultured cells³⁾. The presence of HS in the cellular microenvironment has led to speculation about its possible role in the regulation of cellular growth⁴⁾ and transformation⁵⁾, cell-matrix adhesion³⁾, and cell proliferation processes⁶⁾.

In order to elucidate the structure and function of HSPG, we extracted HS from rat kidney and liver and compared the two molecules by gel chromatography and high-performance liquid chromatography (HPLC).

Materials and Methods

Twenty 13-week-old female Sprague-Dawley rats (Nihon Clea, Tokyo) were used. Glycosaminoglycans (GAG) were extracted from the perfused and defatted kidneys and livers by a combination of alkali treatment and pronase digestion, and they were refined by cetylpyridinium chloride (CPC) and ethanol precipitation. GAG samples were digested with chondroitinase ABC⁷⁾ (Seikagaku Kogyo Co., Tokyo), and HS was then pre-

pared by recovery of the unreacted GAG with CPC. The HS samples from the liver, including an unknown substance, were further purified by Sephadex G-100 chromatography, with 0.5 M ammonium acetate (pH 5.5) as eluent. The fractions that eluted at nearly void volume were collected and desalted by passage over a PD-10 column. The components of GAG were identified by electrophoresis on a cellulose acetate strip. The molecular weight distribution of HS was examined by Sephacryl S-300 HR chromatography. Aliquots of each fraction were used to determine uronic acid by the modified carbazole method of Bitter and Muir⁸⁾. The ratio of glucuronic acid to its epimer, iduronic acid, was determined by the method of Kamada *et al.*⁹⁾ using HPLC with the following additional steps. Hydrolysate (0.05 M HCl, 110 °C, 16 hr) of the HS sample was first treated with the same volume of 50 % triethylamine/ethanol (40 °C, 30 min), evaporated, and dissolved in water. The treated sample solution was prelabeled by a four fold addition of 0.5 % dansylhydrazine/ethanol (containing 0.36% TCA) for 60 min at 40 °C, and the mixture then applied to a Cosmosil NH₂ column (Nacalai Tesque, Kyoto).

Results and Discussion

One-dimensional electrophoretic patterns of the GAG samples from the kidney showed a

Fig. 1 Electrophoresis of heparan sulfates prepared from rat kidney and liver. Electrophoresis was carried out on a cellulose acetate strip in 0.2 M calcium acetate solution at 1 mA/cm for 3 hr. Bands were visualized with 0.5 % Alcian blue 8 GS.

- 1: Standard samples of chondroitin 4-sulfate, dermatan sulfate and hyaluronic acid (from top to bottom)
- 2: Standard samples of heparin (upper), and heparan sulfate from bovine kidney (lower)
- 3: GAG sample from rat kidney
- 4: HS sample from rat kidney
- 5: GAG sample from rat liver
- 6: HS sample from rat liver

distinct band and two moderate bands corresponding to the standard HS, hyaluronic acid, and dermatan sulfate, respectively (Fig. 1, lane 3). These were further confirmed by two-dimensional electrophoresis. The kidney HS sample after enzymatic digestion showed a single band (Fig. 1, lane 4). On the other hand, the GAG sample from the liver showed not only the same bands as those from kidney-HS, hyaluronic acid, and dermatan sulfate—but also an Alcian blue-positive band, which had the slowest rate of migration and corresponded to none of the standards (Fig. 1, lane 5). This large band, which could not be digested with chondroitinase ABC, was obtained from the liver. Therefore the GAG samples from the liver were further purified by gel chromatography. The liver HS sample after enzymatic digestion and

Fig. 2 Gel chromatography of heparan sulfates prepared from rat kidney and liver on a Sephacryl S-300 HR column. The sample (about 300 μ g as glucuronic acid) was applied to a column (0.9 $\times$ 60 cm) equilibrated with 0.5 M ammonium acetate (pH 5.5). Elution was carried out with the same solution at a flow rate of 4.2 ml/hr, and 0.7-ml fractions were collected and analyzed for uronic acid (absorbance at 530 nm). The arrows show the void volume and total volume of the column. —Kidney ····Liver

following gel chromatography showed a single band (Fig. 1, lane 6). There was not much difference between the mobilities of the standard HS and the HS samples from the kidney and liver.

When the molecular weights of the HS samples from the kidney and liver were compared by Sephacryl S-300 HR column chromatography, the molecular weight of the liver sample was significantly larger than that of the kidney one, as demonstrated in Fig. 2. Also the elution profile of the HS sample from the liver was relatively broader than that of the kidney HS, indicating that the distribution in the molecular weights of the former was relatively wider.

HS is a negatively charged polymer made up almost entirely of repeating disaccharide units consisting of glucosamine and uronic acid. The latter may be present as glucuronic acid and its C-5 epimer, iduronic acid; and the iduronic acid residue may be O-sulfated, in this instance at C-2⁽¹⁰⁾. Distinct differences in the ratio of uronic acid epimers

Fig. 3 HPLC of uronic acids in heparan sulfates, as monitored fluorimetrically by precolumn labeling with dansylhydrazine. The sample was applied to a Cosmosil NH₂ column (250×4.6 mm i.d.), and eluted with acetonitrile-0.1 M acetate buffer (pH 5.6, 5/1, v/v) at a flow rate of 1.0 ml/min, with monitoring at 530 nm (emission)/350 nm (excitation).
1: Glucuronic acid 2: Iduronic acid

were detected by HPLC between the HS samples from the kidney and liver (Fig. 3). The ratio of the peak height of glucuronic acid to that of iduronic acid for the kidney sample (14.7) was higher than that for the liver one (7.0). Since a standard sample for free iduronic acid was not available, it was not possible to determine the molar ratio between the two epimers. The difference in the ratio of glucuronic acid to iduronic acid may be the result of the difference in the number of O-sulfated residues in the HS chain.

HS samples from the kidney differed from those from the liver as judged from gel chromatography and the ratio of uronic acid epimers determined by HPLC. Such structural differences observed in HS chains from different tissues may indicate that functional

differences in them exist as well.

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