Tohoku J. exp. Med., 1982, **136**, 61–66

Characteristics of Urinay Glycosaminoglycans Excreted by a Patient with the Hurler–Scheie Compound Syndrome

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Kimura, A., Hayashi, S., Koseki, M. and Tsurumi, K. *Characteristics of Urinary Glycosaminoglycans Excreted by a Patient with the Hurler-Scheie Compound Syndrome.* Tohoku J. exp. Med., 1982, **136** (1), 61–66 —— Glycosaminoglycan isolated from urine of a patient with the Hurler-Scheie compound syndrome consisted of dermatan sulfate (60%), heparan sulfate (34%) and chondroitin sulfate (6%). About 60% of both dermatan and chondroitin sulfates had molecular weight 8,000–10,000, while 95% of the heparan sulfate had molecular weight less than 6,000. The total sulfate content of the glycosaminoglycans increased with decrease in molecular weight. N-sulfate content in the heparan sulfate, however, had no relation to molecular weight, and was 0.33 mole per mole of glucosamine on the average. About 70% of the heparan sulfate with the lowest molecular weight (1,500) were composed of three repeating disaccharide units of heparan sulfate and two acetyl, one N-sulfate and three O-sulfate groups linked to the units. The dermatan sulfate contained 1.0–1.2 moles of sulfate per mole of galactosamine. Of the excess sulfate 45–65% were bound to iduronate residues and the rest to C-6 of N-acetylgalactosamine 4-sulfate residues. Most of the dermatan sulfate (83.2–100%) had nonsulfated iduronic acid at the non-reducing end. This finding is consistent with the defect of iduronidase in this disease. —— mucopolysaccharidosis; Hurler-Scheie compound syndrome; urinary glycosaminoglycan; heparan sulfate; dermatan sulfate

The mucopolysaccharidoses, including the Hurler-Scheie compound syndrome, are caused by the deficiency of specific enzymes for glycosaminoglycan (GAG) degradation, and characterized by the excessive accumulation and abnormal excretion of GAGs. To learn the effect of the enzyme deficiency on the structure of the abnormally excreted GAGs, we attempted to isolate and characterize the urinary GAGs in various types of the mucopolysaccharidosis.

Our previous studies on the urinary GAGs from a patient with the Hunter syndrome (iduronosulfatase deficiency) showed that the dermatan sulfate (DS) and the heparan sulfate (HS) was heterogeneous in molecular weight and sulfate content, and that the DS had an iduronosulfate residue at the non-reducing end, consistent with the defect of iduronosulfatase in that disease (Kimura et al. 1980a, b).

In the present paper we demonstrate the structural features of urinary GAG

Received for publication, March 30, 1981.

excreted by a patient with the Hurler-Scheie compound syndrome (iduronidase deficiency). Like the Hunter syndrome, this disease is characterized by excessive excretion of DS and HS. These two GAGs share α-L-iduronide and α-L-idurono-sulfate linkages.

The clinical features of the patient were previously reported by Kajii et al. (1974).

Experimental

The methods used in this study, unless stated otherwise, were described in our previous papers (Kimura et al. 1980a, b). Briefly, the urinary GAGs were fractionated on a column of Dowex 1-X2 (Cl^-, 200–400 mesh) after removal of salt by passing the urine through Sephadex G-10 gel. Each fraction separated on the column was divided into the main (A) and the tailing (B) parts. They were further fractionated by Sephadex G-50 gel chromatography. Molecular weight was estimated by gel filtration with Sephadex G-50 and G-100 in 1.0 M NaCl. Electrophoresis on a cellulose acetate sheet in 0.075 M barium acetate at 1 mA/cm for 1 hr was carried out to identify the fractionated GAGs. To elucidate its structure, the DS was digested with chondroitinase (Chase) ABC. The resulting disaccharides were identified by chemical and enzymatic examinations after separation from one another by Dowex 1 column chromatography.

Results and Discussion

Fractionation and identification of urinary GAG. The distribution of GAG among the fractions separated on a column of Dowex 1 with 0.5, 0.8, 1.0, 1.25, 1.5 and 2.0 M NaCl was 10.9, 25.3, 11.5, 12.5, 35.6, and 4.2% of the total, respectively. They were fractionated into 36 fractions by the procedure described in Experimental. Some of these fractions contained two or three different kinds of GAG, which were separated and quantitatively determined by the electrophoretic method. The results showed that 60% of GAG was DS, 34% HS and 6% chondroitin sulfate (CS). The data of chemical and electrophoretic analyses of ten major fractions are listed in Table 1.

Table 1. *Analysis and identification of fractionated GAGs*

Fraction	Yield* (mg)	Uronic acid†	Sulfate†		Acetyl†	M. W. ($\times 10^3$)	Identification‡
			Total	N-S			
0.8A1	81.9	1.23	0.65	0.25	0.95	5.7	HS (DS)
0.8A2	29.4	1.36	0.76	0.30	0.85	3.8	HS
0.8A3	82.1	1.31	1.14	0.30	0.77	1.5	HS
1.0B4	45.8	1.14	1.32	0.37	0.74	1.5	HS (DS)
1.25A4	44.9	0.95	1.16	0.11	0.96	3.5	DS: HS=2: 1
1.25B3	37.0	0.94	1.18	0.09	0.89	3.8	DS (CS, HS)
1.5A1	247.2	0.97	1.00	0.04	0.94	9.9	DS (CS)
1.5A2	103.0	0.90	1.04	0.04	0.93	5.2	DS (CS)
1.5B1	80.0	0.83	1.03	0.03	0.93	9.2	DS (CS)
2.0A1	50.0	0.88	1.16	0.06	1.15	9.2	DS (CS)

* About 75% of the total GAG obtained from the urine specimen (4.0 liters) was taken up in this table.

† Molar ratio with hexosamine as 1.00.

‡ The GAG in parentheses is less than 10% of the total amount of GAG in the fraction.

The preparation obtained in this experiment contained small molecular GAG which could not be precipitated with quaternary ammonium ion (QAI) in addition to QAI-precipitable GAG. When GAG was isolated from urine of the patient by precipitation with QAI, the proportion of DS increased to around 80% and that of HS decreased to 10%.

The patient's daily excretion of the QAI-precipitable GAG was about 30 mg as glucuronic acid (Kajii et al. 1974). The value is ten-fold higher than the average value given by healthy children of the same sex and age.

Distribution of molecular weight of GAG. The molecular weight of GAG obtained in this experiment ranged from 1,500 to 11,000. The DS and CS showed similar distribution patterns to each other. The pattern of the HS, however, was different from them, shifted to lower region (Fig. 1). About 60% of the DS and the CS had molecular weight 8,000–10,000, whereas 95% of the HS had molecular weight less than 6,000. Similar results were reported on the HS obtained from urine of the Hunter syndrome (Kimura et al. 1980a).

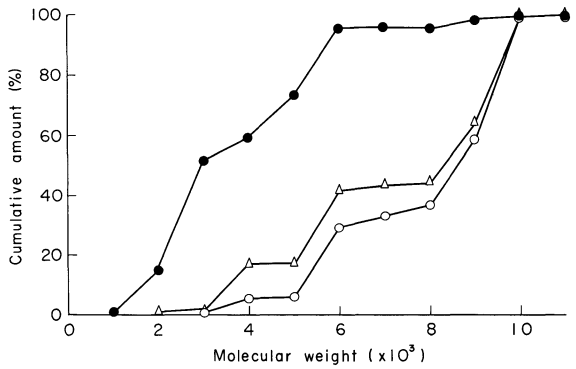

Fig. 1. Distribution of molecular weight of GAG.
●—●, HS; △—△, DS; ○—○, CS.

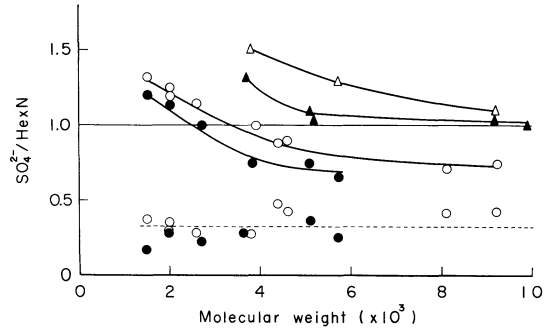

Fig. 2. Relation of sulfate content to molecular weight of DS and HS.
Solid line: total sulfate of 0.8 (●), 1.0 (○), 1.5 (▲) and 2.0 (△) M fractions. Dotted line: N-sulfate of 0.8 (●) and 1.0 (○) M fractions.

Structural characteristics of HS. Fig. 2 shows the relation of sulfate content to molecular weight of the DS and the HS fractions. Both DS and HS of lower molecular weight contained a larger amount of total sulfate. N-sulfate content in the HS, however, had no relation to molecular weight, and the average value was 0.33 mole per mole of glucosamine.

The chemical analysis suggested that the HS with the lowest molecular weight 1,500 (fractions 0.8A3, 1.0B4 and 1.25A5) was composed of three repeating units, $[UA\text{-}GlcN]_3$, and various numbers of sulfate and acetyl groups as shown in Table 2. The table also includes three presumed hexasaccharides A, B and C. Fraction 0.8A3 might contain A and B in a ratio of 1:1. Fraction 1.25A5 seemed to be a mixture of B and C (4:1). Thus about 70 per cent of the HS with the lowest molecular weight were shown to be identical with the hexasaccharide B in their chemical components.

Chemical characteristics of DS. To investigate the chemical structure of DS, four major DS-fractions (1.5A1 1.5A2, 1.5B1 and 2.0A1) were digested to the disaccharide units with Chase ABC after removal of contaminating CS by digestion with Chase AC followed by gel filtration through Sephadex G–15. The resulting

TABLE 2. *Acetyl and sulfate contents of HS-hexasaccharides**

Hexasaccharide	Yield† (mg)	Sulfate		Acetyl
		Total	N-S	
Isolated				
0.8A3	82.1	3.6	0.5	2.7
1.0B4	45.8	4.0	1.1	2.2
1.25A5	8.2	4.2	1.2	1.8
Presumed				
A		3.0	0	3.0
B		4.0	1.0	2.0
C		5.0	2.0	1.0

* Molar ratio with glucosamine as 3.00.
† Obtained from 4.0 liters of the urine specimen.

TABLE 3. *Structures of disaccharide units and their proportions in DS molecules*

Fraction	Structure*	Amount (%)			
		1.5A1	1.5A2	1.5B1	2.0A1
A′	IdUA-GalNAc	0.25	0.45	0.26	0.25
B	⊿UA-CalNAc	1.97	3.37	1.84	1.53
C	IdUA(S)-Ga1NAc	0.03	0.13	0	0.03
E′	IdUA-GalNAc-4S	4.00	7.53	4.90	4.06
F	⊿UA-GalNAc-4/6-S	89.34	80.77	87.51	75.88
G	IdUA(S)-GalNAc-4-S	0.60	1.03	0	0.84
I	⊿UA-GalNAc-4,6-diS	1.89	2.76	1.95	10.06
J	⊿UA(S)-GalNAc-4-S	1.92	3.96	2.07	7.34

* Abbreviations: IdUA, induronic acid; ⊿UA, unsaturated glucuronic acid; GalNAc, N-acetylgalactosamine; S, sulfate.

disaccharides of DS were fractionated on a column of Dowex 1-X2 (Cl^-, 200–400 mesh). Eight fractions were obtained in the ratios shown in Table 3.

The disaccharides C, F, G, I and J were also isolated from the digestion products of the DS of the Hunter disease, and their structures were previously identified as those shown in the table (Kimura et al. 1980b). The fraction F was a mixture of ⊿UA-GalNAc-4-S and ⊿UA-GalNAc-6-S. The amount of ⊿UA-GalNAc-6-S (10–13%) was calculated on the basis of the Morgan-Elson values determined before and after treatment with chondro 4-sulfatase.

The elution volumes of the fractions A′, B and E′ were practically the same as that of fractions A, B and E, respectively. The fractions A, B and E were derived from low-sulfated chondroitin 4-sulfate and their structures were shown in our previous paper (Kimura et al. 1980b). The ratios of uronic acid value determined by the Dische method to that by the Bitter-Muir method were about 0.9 for the fractions A and E, and about 0.4 for the fractions A′ and E′, suggesting that the fractions A and E have GlcUA and the fractions A′ and E′ have IdUA as their uronic acid components. The fraction E′ lost its sulfate residue and decreased its elution volume to the same extent as that of A′ on the column chromatography with Dowex 1 after treatment with chondro 4-sulfatase.

IdUA-GalNAc (A′) and GlcUA-GalNAc (A) were separated from each other by chromatography on a Dowex 1-X2 column (formate form, 200–400 mesh, 0.5× 100 cm, eluted with 0.3 M formic acid at a flow rate 0.5 ml a tube/20 min). The chromatography showed that the pooled fraction A′ contained 96.3 per cent of IdUA-GalNAc and 3.7 per cent of GlcUA-GalNAc.

In the urinary DS of the Hunter disease, about 90 per cent of the excess sulfate groups were linked to the IdUA residues (Kimura, et al. 1980b). On the other hand, the value decreased to 45–65 per cent in the DS obtained from the urine of the present patient with the Hurler-Scheie compound syndrome. The total sulfate content also decreased in the DS of this patient. These results are compatible with the finding by Bach et al. (1973) that the proportion of unsaturated, disulfated disaccharides produced from GAG of Hunter fibroblasts by Chase ABC was reduced by one-third after treatment with the Hunter corrective factor (iduronosulfatase). These results suggest that iduronosulfatase releases sulfate groups linked not only to nonreducing terminal IdUA but also to some internal IdUA residues.

The "disaccharide analysis" revealed that there were two kinds of DS in urine of the present patient, one had IdUA and the other S-IdUA at their non-reducing termini. The former (fractions A′+E′) amounted to 83.2 to 100 per cent of the total molecules (fractions A′+E′+C+G). The result is consistent with the defect of iduronidase in this disease.

Acknowledgments

We wish to thank Dr. I. Matsuda, Department of Pediatrics, Faculty of Medicine, Kumamoto University, for supplying us with the urine specimen of the patient. This work was supported in part by a grant from the Ministry of Education, Science and Culture, Japan.

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