

Communications to the Editor

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ANALYSIS OF CHONDROITIN SULFATES IN HUMAN URINARY TRYPSIN INHIBITOR

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A proteoglycan (PG) was separated from human urine by ion chromatography and gel filtration. With our sensitive determination method, the PG (M.W. Ca. 67,000) was found to contain low-sulfated chondroitin 4-sulfate (Ch4S). The PG inhibited trypsin and had the same chromatographic behavior as human urinary trypsin inhibitor (HUTI), which also contained the low-sulfated Ch4S. From these results, it was concluded that the PG and HUTI were the same substance. The difference between individuals in the composition of the low-sulfated Ch4S in HUTI was also examined.

KEYWORDS — chondroitin sulfate; glycosaminoglycan; proteoglycan; urinary trypsin inhibitor

Glycosaminoglycans (GAGs) in human urine have been studied for many years. Recently, we separated human urinary proteoglycan (PG) by ion chromatography and gel-filtration as follows. GAGs were collected from 100 ml of normal human urine according to a method for the formation of cetylpyridinium chloride (CPC) complexes. The crude GAGs were applied to a DEAE-cellulose column (Fig. 1). The fractions

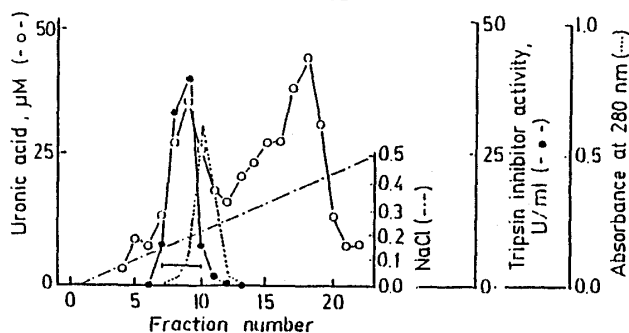


Fig. 1. Elution Profile of GAGs on DEAE-Cellulose Column

A DEAE-cellulose column (1 cm i.d. x 13 cm) was equilibrated with 0.1 M Tris-HCl buffer (pH 7.8). Elution was performed by a 0-0.1 M NaCl gradient in the same buffer. Flow rate was 12 ml/h. Fractions of 5 ml were collected.

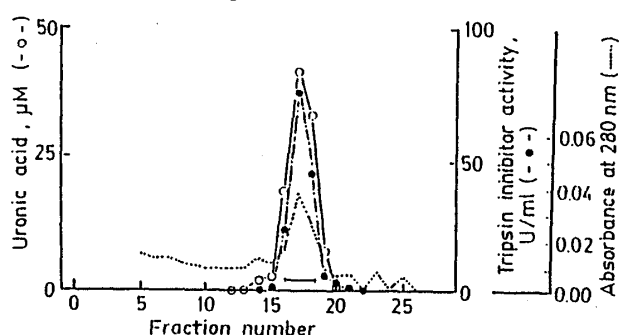


Fig. 2. Elution Profile of the Sample Described in Fig. 1

A Sephadex G-100 column (2.2 cm i.d. x 81 cm) was equilibrated with 0.1 M Tris-HCl buffer (pH 7.4) containing 0.2 M NaCl. Flow rate was 12 ml/h. Fractions of 7 ml were collected.

corresponding to the first peak of uronic acid (Fractions 7-10 in Fig. 1) were combined, dialyzed against distilled water, and lyophilized. The residue was gel-filtered on a Sephadex G-100 column (Fig. 2). A major peak which corresponded to the PG of 67,000 M.W. (PG-I) was obtained. Chondroitin sulfate (ChS) in PG-I was analyzed according to the procedure established previously by us,¹⁾ and the analytical result indicated that PG-I contained low-sulfated chondroitin 4-sulfate (Ch4S) as shown in Fig. 3.

Balduyck et al.²⁾ reported that human urinary trypsin inhibitor (HUTI) has a ChS chain. So HUTI was isolated according to Maehara's procedure³⁾ and compared with PG-I. Both HUTI and PG-I showed the same chromatographic or electrophoretic behavior and trypsin inhibitor activity. Furthermore, HUTI contained the low-sulfated Ch4S with the same composition as PG-I, as shown in Fig. 3.

From the results, it was concluded that PG-I and HUTI were the same substance.

Next we examined the composition of the ChS in the HUTI from several individuals. The relative amounts (%) of Δ Di-0S and Δ Di-4S from the HUTI of 3 males and 4 females (20-24 ages) were almost the same, as shown in Table I.

It is interesting to know if this composition differs with diseases or aging.

It has been hypothesized that HUTI originates from inter- α -trypsin inhibitor by limited proteolysis, but there is much controversy about this. So we are also undertaking a study of the low-sulfated Ch4S in plasma.

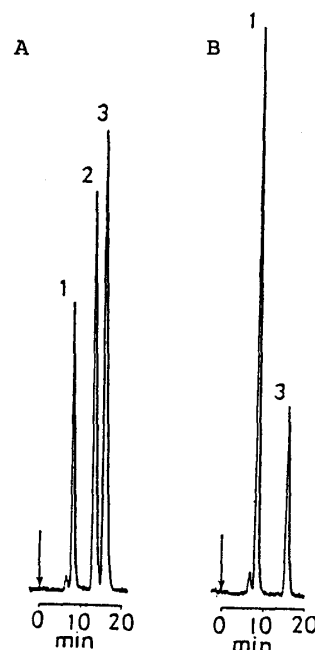


Fig. 3. Chromatograms of Unsaturated Disaccharides Produced from the PG-I or HUTI

A: Standard, B: PG-I or HUTI
1: Δ Di-0S, 2: Δ Di-6S, 3: Δ Di-4S

Table I. Composition of Unsaturated Disaccharides Produced from Low-Sulfated Ch4S in HUTI after Digestion with Chondroitinase ABC

Case No.	Sex	Unsaturated disaccharide		
		Proportion (%)		
		Δ Di-0S	Δ Di-4S	Δ Di-6S
1	M (*)	66	34	N.D.
2	M	66	34	N.D.
3	M	62	38	N.D.
4	F	69	31	N.D.
5	F	65	35	N.D.
6	F	66	34	N.D.
7	F	60	40	N.D.

(*) M: Male, F: Female.

Abbreviations used: Δ Di-0S, 2-acetamido-2-deoxy-3-O-(β -D-glucosyl-4-enepyransyluronic acid)-D-galactose; Δ Di-6S, 2-acetamido-2-deoxy-3-O-(β -D-glucosyl-4-enepyransyluronic acid)-6-O-sulfo-D-galactose; Δ Di-4S, 2-acetamido-2-deoxy-3-O-(β -D-glucosyl-4-enepyransyluronic acid)-4-O-sulfo-D-galactose.

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