Glycosaminoglycans of Normal Human Prostate[1] (36398)

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In vivo, glycosaminoglycans (GAG) (acid mucopolysaccharides) may influence directly the concentration of prostatic zinc. This concept is based on the results of histochemical (1, 2), radioautographic (2), and ^{65}Zn-binding experiments (2, 3) with human prostatic tissue which disclosed that more than 90% of the total ^{65}Zn is bound to GAG and less than 10% is bound to protein.

This investigation was designed to determine the amount and types of GAG present in the normal human prostate.

Materials and Methods. Five grossly and histologically normal adult human prostate glands obtained at autopsy were studied (Table I). The tissue was blended in acetone, extracted with methanol–chloroform (1:1, v/v), and then dried *in vacuo*. For the digestion of the tissue, the method of Scott (4) was modified as follows: 1 g of dried tissue was suspended in 10 ml of 0.1 *M* phosphate buffer (pH 6.8) containing 0.05 *M* ethylenediaminetetraacetic acid and 0.01 *M* cysteine. The suspension was degassed under reduced pressure, and after addition of 10 mg of papain (PAP OJA, 13.3 units/mg, Worthington Biochemical Corp., Freehold, NJ), it was incubated at 60° for 16 hr with constant stirring. The digest was cleared by centrifugation and the residue was washed with water once. The combined supernatants were dialyzed against cold distilled water and cleared again by centrifugation. Aliquots of this crude extract were used for the determination of hexuronic acid (5), for chromatography on Dowex-1 X2 columns (200–400 mesh, Cl^- form) (6), and for ethanol precipitation of GAG.

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Chromatography of the extract on Dowex 1 X2. After application of the sample, the column (7 × 150 mm) was washed with water and then eluted with solutions of increasing NaCl concentration (Table II). On occasion, the fractionation of GAG from additional normal prostates was modified as indicated in Table II. An aliquot of each effluent was spotted on Whatman No. 1 chromatography paper, dried and stained with 0.1% alcian blue as described below. Elution with each salt solution was continued until GAG could no longer be detected. In addition, 20 ml of effluent was collected for the 2.0 *N* HCl and 2.0 *N* NaOH fractions. Each fraction was dialyzed and concentrated to a suitable volume under reduced pressure. Aliquots were used for measurements of hexuronic acid (5), for electrophoresis on cellulose acetate membranes (7), and in 7.5% polyacrylamide gel (8), and for incubation with testicular or streptococcal hyaluronidase (9).

Electrophoresis was performed with the Beckman Microzone system at 9.0 mA for 35–45 min in a solution of 0.1 *N* KOH in 1.7 *N* acetic acid (pH 3.2). Samples were adjusted to contain 1–2 μg of hexuronic acid/μl. The membranes were stained in 0.1% alcian blue in 5% acetic acid (10) for 20 min, destained in 5% acetic acid, dehydrated in ethanol for 1 min, cleared in cyclohexanone–ethanol (30:70, v/v) for 1 min, and then dried at 105° for 15 min (7). Mobilities of GAG were compared with reference standards, kindly supplied by Dr. J. A. Cifonelli (The University of Chicago, Chicago, IL).

Samples of the chromatographic fractions containing 5–20 μg hexuronic acid were digested for 15 hr with testicular hyaluronidase (T-HAase) (HSEP OHA, 4000 units/mg,

TABLE I. Age of Patients, Time of Removal After Death; Size, Dry Weight as Percentage of Wet Weight, and Hexuronic Acid Content of the Prostates.

No.	Age	Time of removal (hr)	Wt of prostate (g)	Wet wt of tissue (g)	Dry wt[a] (%)	Hexuronic acid[b] (mg/g of dry wt)
1	44NM	6	20.8	17.8	14.7	2.15
2	34WM	10	19.9	12.0	14.8	3.20
3	41NM	4	22.2	18.2	14.0	1.17
4	23NM	3	19.7	14.8	13.6	12.09
5	45WM	8	18.4	10.2	14.7	5.53

[a] 14.2 SD ± 1.11.
[b] 4.83 SD ± 4.04.

Worthington Biochemical Corp., Freehold, NJ) (9). Digestion with streptococcal hyaluronidase (S-HAase) (42 units/mg, provided by Lederle Labs., Pearl River, NY) was conducted under similar conditions (9). The digests were dialyzed, lyophilized, and then examined by membrane and gel electrophoresis.

Ethanol precipitation of GAG. Aliquots of the crude extracts were precipitated with absolute ethanol containing 1% potassium acetate (9); one fraction was obtained at 50% ethanol concentration and a second one at 67% ethanol concentration, after storing the fraction overnight at 4°. The precipitates were washed with absolute ethanol twice, dried *in vacuo*, and weighed. These precipitates were dissolved in water and aliquots were used for the determination of hexuronic acid by the borate–carbazole method (5), hyaluronic acid (HA) by the Morgan-Elson reaction cited in (9) after hydrolysis with S-HAase (9, 15), sulfoaminohexose (SAH) by the nitrous acid method (11), chondroitin-4-sulfate (CS4), chondroitin-6-sulfate (CS6), and dermatan sulfate (DS) by the enzymic Method I (9). Dermatan sulfate was also determined: (a) colorimetrically with the periodate–Schiff method (12), and (b) with the Morgan-Elson reaction following hydrolysis with T-HAase, dialysis and incubation of the nondialyzable material with chondroitinase ABC and chondro-4-sulfatase (Miles Labs., Inc., Kankakee, IL) (9). The absorbancies of samples were measured in 1 cm cuvettes with the Beckman DU-2 spectrophotometer.

Results. The average dry weight obtained

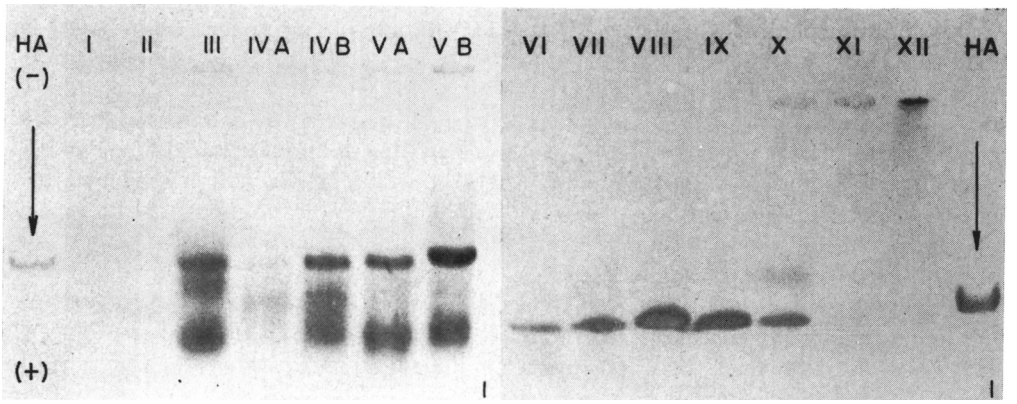

FIG. 1. Electrophoresis of Fractions I–XII (Table II) and standard hyaluronic acid (HA) on cellulose membrane in acetate buffer (pH 3.2) at 9.0 mA constant current for 35 min (Fractions I–VB) and 45 min (Fractions VI–XII). Stained with alcian blue. Direction of migration is indicated by the arrow.

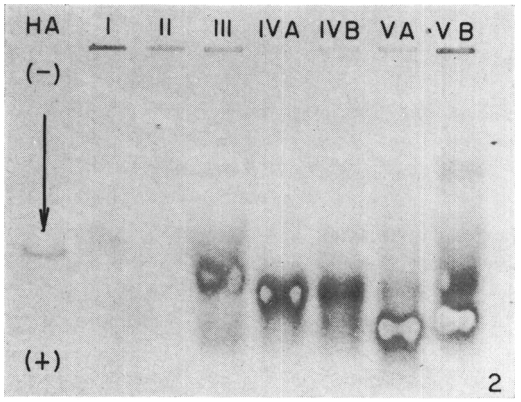

FIG. 2. Electrophoresis of Fractions I–VB (after digestion with testicular hyaluronidase) for 35 min. Conditions were similar to Fig. 1. Note: The light blotches are areas of flaking due to overloading of sample. Membranes were overloaded with each sample to show the disappearance of the band migrating like hyaluronic acid (TA).

after extracting 5 normal human prostate glands with acetone and methanol–chloroform was 14.2 ± 1.11% of the wet weight (Table I). The crude extracts of these tissues, obtained after digestion with papain, contained 4.83 ± 4.04 mg of hexuronic acid/g of dried tissue by the borate–carbazole method (5).

The crude extracts of 3 prostates were separated into 5, 8, and 12 chromatographic fractions (Table II); the total recoveries of GAG were 62, 80, and 72%, respectively, of the amounts of GAG applied to the columns based on the borate–carbazole method for total hexuronic acid (5). These recoveries agree with those reported for other tissues (6, 13). There was a wide variance in the percentage of hexuronic acid in Fractions I and V of the 3 prostates. Fraction I was syrupy, and amber colored upon lyophilization. No GAG were detectable with alcian blue on the membrane electropherogram in Fractions I and II even at concentrations 100 times higher than those necessary to visualize standard GAG (Fig. 1). In Fractions III to X, a band migrating like HA was present. The component of this band could be depolymerized with T-HAase (Fig. 2). S-HAase depolymerized this component only in chromatographic Fractions III and IV A (Fig. 3). Unlike T-HAase (Fig. 2), S-HAase did not abolish other bands nor produce new bands (Fig. 3).

Figure 1 shows that chromatographic Fractions III to V B, eluted with 0.8–2.0 *M* NaCl and representing 70–85% of the total prostatic GAG (Table II), contained GAG with mobilities greater than that of HA. This elution pattern is similar to that of placenta (6) and normal human urine (14).

Precipitation of GAG with ethanol containing 1% potassium acetate produced a fibrous-like precipitate (FP), which formed immediately in 50% ethanol, and a flocculent precipitate (FPS), which formed in 67% ethanol. The average yields per gram of dried tissue from 2 prostates were: FP, 8.59 mg; FPS, 11.6 mg; and the hexuronic acid contents (5) were 5.00 mg and 3.81 mg, respectively. GAG were concentrated mostly in the FPS precipitate. Table III shows the amounts of the various GAG present in these 2 ethanol fractions. Both fractions contained large amounts of protein and nucleic acid (Table IV).

According to the colorimetric method (12), there were 0.64 and 3.50 mg of DS/g of dried tissue in FP and FPS, respectively (Table III). However, when DS was measured using enzymes according to Method I and II (9), the results were too low. Therefore, 2.5× the amounts of chondroitinases and chondrosulfatases as recommended in Ref. 9 were used and the time of incubation was increased from 30 min to 1.5 hr. The

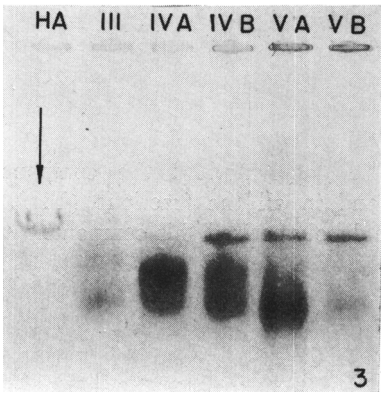

FIG. 3. Electrophoresis of Fractions III–VB (after digestion with streptococcal hyaluronidase) for 35 min. Conditions were similar to Fig. 1.

TABLE II. Hexuronic Acid in GAG Fractions[a] Obtained from Columns (7 × 150 mm) of Dowex-1 X2 (200–400 mesh, Cl^- form).

Fraction	Eluent	Hexuronic acid[b] (%)		
		b	c	d
I	H_2O	6.2	1.8	12.6
II	0.2 *M* NaCl	—	9.6	12.5
III	0.8 *M* NaCl	43.5	15.2	29.0
IV A[c]	1.4 *M* NaCl	—	22.8	24.3
IV B	1.4 *M* NaCl	43.6	11.7	8.3
V A	2.0 *M* NaCl	—	18.0	3.7
V B	2.0 *M* NaCl	—	15.6	5.1
VI	4.0 *M* NaCl	5.2	0.5	0.6
VII	4.0 *M* NaCl (0.01 *N* HCl)	—	—	0.5
VIII	2.0 *N* HCl	—	1.6	0.6
IX	H_2O	—	—	0.6
X	2.0 *N* NaOH	1.6	2.9	0.7
XI	2.0 *N* NaOH[d]			0.7
XII	H_2O			0.8

[a] Obtained from 3 normal human prostate glands b, c, and d.

[b] Determined by the borate–carbazole method (5). Measurements of absorbancies were significant to 3 significant figures. % was rounded to the nearest tenth only after the final calculation.

[c] Fractions IV and V were arbitrarily divided into the front (A) and tail (B) moieties.

[d] Collected at intervals of 2 days.

result was 0.78 mg of DS in FP and 3.88 mg in FPS (Table III). These values being in agreement with those obtained by the colorimetric method, subsequent incubations utilizing this modification offered maximal and consistent results.

Discussion. Membrane electrophoresis has shown the presence of a band migrating like HA in Fractions III to X (Fig. 1). Although T-HAase depolymerized the component of this band in all the fractions (Fig. 2), S-HAase depolymerized this component only in Fractions III and IV A (Fig. 3). Thus, on the basis of these results, the band present only in Fractions III and IV A is considered to be HA (15). The chemical nature of the HA-like component found in Fractions IV B to X is not yet known; however, it appears to be an inhibitor of chondroitinase ABC because this mixture of enzymes would work only after the fractions were incubated with T-HAase.

Spectrophotometric measurements disclosed that significant amounts of protein and nucleic acid were present in FP and FPS (Table IV). Since the borate–carbazole method (5) is significantly affected by protein and other substances (16), the total amount of hexuronic acid measured by this method may represent an over estimation. Thus, the hexuronic acid content was 58% in FP and 33% in FPS (Note *a* of Table III); however, the GAG content was 13% in FP and 59% in FPS (Table III). The wide variance in the hexuronic acid content of the prostate glands listed in Table I may be due to a combination of individual differences and the varying amounts of substances which affect the borate–carbazole method (5).

The concentration of DS in the normal human prostate was more than the sum of the other GAG (Table III).

The results obtained with histochemical techniques indicate that the normal prostatic secretion does not contain strongly acidic GAG (17, 18). Thus, it is possible that the strongly acidic sulfated GAG, especially DS,

TABLE III. GAG of the Normal Human Prostate (mg/g of dried tissue).

GAG	FP[a]	FPS[a]
DS[b]	0.78	3.88
DS[c]	0.64	3.50
HA	0.33	1.35
CS4	0.12	0.62
CS6	0.02	1.28
SAH[d]	—	0–0.08

[a] Average of 2 prostates. FP (8.59 mg/g of dried tissue; hexuronic acid, 5.00 mg), fibrous-like precipitate, formed in 50% ethanol. Flocculent precipitate [(FPS) 11.6 mg/g of dried tissue; hexuronic acid, 3.81 mg] formed in 67% ethanol.

[b] Enzymic method. Samples were dialyzed after 15-hr digestion [according to Method II of Ref. (9)] with T-HAase and then digested with 2.5X the amount of chondroitinase ABC and chondro-4-sulfatase for 1.5 hr. The product was determined by the Morgan-Elson reaction (9).

[c] Colorimetric method (12).

[d] No attempts were made to measure acetyl aminohexose or aminohexose present in the sample.

TABLE IV. Absorbancies of FP and FPS per Gram of Tissue at 235, 260, and 280 nm and the Ratios of These Absorbancies.

Ethanol precipitates	Absorbancies/g of tissue			Ratios of absorbancies	
	235	260	280	260/280	260/235
FP[a]	52	101	56	1.8	1.9
FPS[a]	46	82	44	1.9	1.8

[a] Average of 2 normal human prostates (see Table III).

present in the crude prostatic extract are derived from the stroma of the prostate rather than from the epithelial cells and the secretion.

Summary. Crude extracts of normal human prostate glands obtained after papain digestion contained 4.83 ± 4.04 mg of hexuronic acid/g of dry tissue. Aliquots of the extracts were fractionated on columns of Dowex-1 X2 with solutions of increasing NaCl concentrations. Electrophoresis of the chromatographic fractions showed the presence of a band migrating like hyaluronic acid (HA) in Fractions III to X. The component of this band in Fractions III and IV A is considered to be HA because it was depolymerized by streptococcal hyaluronidase. The corresponding band in Fraction IV B to X is referred to the HA-like component because it was depolymerized by testicular hyaluronidase but not by streptococcal hyaluronidase.

Additional aliquots of the crude extracts were separated into fibrous-like (FP) and flocculent (FPS) precipitates by the addition of ethanol. FPS contained 5 times more GAG than FP. In both precipitates, dermatan sulfate was the most abundant GAG. Spectrophotometric measurements indicated the presence of protein and nucleic acid in FP and FPS.

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