Contents and compositions of glycosaminoglycans in different sites of the human hip joint cartilage

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SUMMARY The distribution of glycosaminoglycans (GAGs) in different functional regions (weight-bearing and nonweight-bearing portions) of the human hip joint cartilage was studied. The results obtained were as follows: (1) Weight-bearing cartilage contains larger amounts of GAGs than nonweight-bearing cartilage. (2) Weight-bearing cartilage contains keratan sulphate in higher ratio to chondroitin sulphate than nonweight-bearing cartilage. (3) The differences in content and composition of GAGs between the weight-bearing and nonweight-bearing portions are more pronounced in the femoral head than in the acetabulum. The preliminary analyses showed that the chondroitin sulphate from the acetabular cartilage contained exclusively 6-sulphated disaccharide units and there was some heterogeneity in keratan sulphate.

The distributional features of the glycosaminoglycans (GAGs) in normal articular cartilage have been reported from the tangential and topographical points of view. Most of these studies showed that in mature cartilage both the concentration of total GAG and proportion of keratan sulphate increased with the depth of the tissue.¹⁻⁹ The tangential distribution of GAGs in the cartilage was found to produce a gradual increase of internal osmotic pressure, thus imparting to the cartilage the mechanical function of resiliently resisting a force applied to the joint.^{10, 11} This hypothesis seems to be supported by the evidence that the compressive stiffness depends on the concentration of GAGs, especially keratan sulphate.¹²

Previous studies therefore suggest there is close relation between the content and composition of GAGs and the function of the joint cartilage on loading. However, there are only a few reports on the topographical distribution of GAGs over the articular cartilage in relation to the loading area. Moreover the results of these studies have not been unanimous, chiefly because of differences in sampling and analytical procedures.

The purpose of the present investigation was to clarify the differences in the content and composition of GAGs in the weight-bearing and nonweight-bearing regions of the 2 opposing cartilages in the hip joint. In our study a high-porous anion-exchanger

was used for the separation analysis of GAGs, and a relatively large amount of material was obtained in an attempt to characterise it more accurately. Sampling regions of the cartilage have been determined in accordance with weight-loading conditions,¹³ and each GAG concentration was measured by analysis of dry specimens of the full-thickness cartilage in the area.

Materials and methods

Hip joint. The human femoral heads and acetabula obtained from 10 fresh cadavers were stored at -20°C until use. The ages of the subjects ranged from 47 to 75 years, average 60.4. All cartilage specimens were apparently normal apart from the expected aging process.

Preparation of the dry cartilage. After the joint surface was washed with water specimens of weight-bearing cartilage were taken by curettage from the superior portion of the femoral head and of nonweight-bearing cartilage from the inferior portion below the fovea (Fig. 1, left). The acetabulum was divided into 5 pieces¹⁴ by radial cuts (Fig. 1, right) and the cartilage was removed from the areas 2, 3, and 4 as weight-bearing portion and from areas 1 and 5 as nonweight-bearing portion. The specimens of cartilage were cut into small pieces, defatted and dehydrated in acetone, and dried in vacuum over P₂O₅ at 80°C.

Preparation of crude GAGs. The weighed dry cartilage was swelled in water and denatured by boiling. The denatured cartilage was suspended in 0.1 M

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Fig. 1 Schematic drawing of the hip joint cartilage. The dotted areas indicate the sites of specimens removed for investigation.

Tris-HCl buffer, pH 8.0, with 0.01% calcium acetate, and incubated with 30 μ g of pronase E (Kaken Kagaku Co., Tokyo) per mg of dry cartilage at 37°C overnight. The digest was adjusted to be 0.5 N at the final concentration and kept at 37°C for 1 hour. After neutralisation with acetic acid the solution was filtered through Celite and concentrated. To this solution several volumes of ethanol containing 0.3% sodium acetate were added, and the precipitate formed was dissolved in a small amount of water and reprecipitated. Crude GAGs were then collected by centrifugation.

Fractionation of GAGs. Crude GAGs were dissolved in water and applied on the column of high-porous anion-exchange resin (Diaion HPA-10, Mitsubishi Kasei Co., Tokyo). The columns were eluted with water, 0.2 M, 0.5 M, 1 M, 1.5 M, and 3 M NaCl in sequence.

Analytical methods. Uronic acid was determined by the carbazole method of Bitter and Muir¹⁵ with glucuronic acid as standard. Hexose was determined by the anthrone method¹⁶ with galactose as standard. Hexosamine was assayed by using an amino acid AutoAnalyzer (Hitachi KLA-5) on samples hydrolysed with 4 N HCl at 100°C for 18 hours in a vacuum. Infrared spectrophotometry was carried out with a JASCO IR-S instrument by the KBr pellet technique. Electrophoresis was performed on cellulose acetate strips in 0.1 M HCl. GAG standards

and chondroitinase-AC (CHase-AC II) were obtained from Seikagaku Kogyo Co., Tokyo. Chondroitinase digestion was carried out in 0.04 M Tris-HCl buffer (pH 6.4), and the unsaturated disaccharides formed were separated by paper chromatography according to Saito *et al.*¹⁷ After elution from the filter paper the disaccharides were analysed by carbazole reaction. Gel filtration was performed on a column (1.5 $\times$ 93 cm) of Sephadex G-200 with aqueous 0.5 M NaCl.

Results

PRELIMINARY EXPERIMENTS

The chromatographic behaviour of GAGs prepared from the weight-bearing cartilage was investigated on a preliminary basis, and the results are shown in Fig. 2. Uronic acid and hexose contents were determined as indices for the estimation of GAGs. Galactosamine was eluted only in 1 M and 1.5 M NaCl fractions, while glucosamine and hexose were eluted over all fractions. The ratio of galactosamine in 1 M to 1.5 M fractions was considerably higher in the femoral head than in the acetabulum. A large amount of hexose observed in 0.2 M NaCl fractions showed no metachromasy and it was excluded.

All fractions were analysed on cellulose acetate electrophoresis before and after chondroitinase digestion (Fig. 3). According to the mobility and

Fig. 2 Sugar analyses of GAG fractions obtained from the anion exchange column.

Fig. 3 Cellulose acetate electrophoresis of GAG fractions before and after chondroitinase digestion. About 20 μ g of aliquots were applied on cellulose acetate and subjected to electrophoresis for 2 h at 1 mA/cm in 0.1 M HCl. HA=Hyaluronic acid, C6S=chondroitin 6-sulphate, CHase=chondroitinase.

susceptibility to the enzyme the main GAGs in these fractions were identified as hyaluronic acid in 0.5 M fraction, chondroitin sulphate in 1 M and 1.5 M fractions, and keratan sulphate in 1.5 M and 3 M fractions. Although the 1 M fraction contained GAGs rich in hexose and glucosamine, it showed only weak metachromasy after digestion by chondroitinase. Therefore all amounts of hexose did not necessarily account for the component of keratan sulphate.

The infrared spectra of 1.5 M and 3 M fractions of the acetabulum showed a pattern having absorption

bands at 1000 cm^{-1} , 820 cm^{-1} , and 775 cm^{-1} similar to those of standard chondroitin 6-sulphate and keratan sulphate from shark (Fig. 4).

Chondroitin sulphate from the acetabulum produced 86% of Δ Di-6S by chondroitinase digestion, suggesting that it belongs to chondroitin 6-sulphate. There was little variation in the disaccharide ratio of chondroitin sulphate over the entire area in the acetabulum (Table 1).

The gel chromatographic behaviour of keratan sulphate and chondroitin sulphate indicated that chondroitin sulphate might be of larger molecular

Fig. 4 Infrared spectra of GAG fractions obtained from acetabulum cartilage. (1) 1.5 M Fraction. (2) Standard chondroitin 6-sulphate. (3) 3 M Fraction. (4) Standard keratan sulphate.

Table 1 Molar ratio of the unsaturated disaccharides obtained by digestion with chondroitinase-AC

Sampling areas of acetabulum					
	1	2	3	4	5
Δ Di-4S	13.1 $\pm$ 2.2	11.3 $\pm$ 0.5	12.6 $\pm$ 1.2	17.1 $\pm$ 3.1	12.6 $\pm$ 1.7
Δ Di-4S+ Δ Di-6S					

Values are means $\pm$ SD (n = 5).
 Δ Di-4S: 2-acetamido-2-deoxy-3-O-(β -D-gluc-4-enepyranosyluronic acid)-4-O-sulfo-D-galactose.
 Δ Di-6S: 2-acetamido-2-deoxy-3-O(β -D-gluc-4-enepyranosyluronic acid)-6-O-sulfo-D-galactose.

size than keratan sulphate, and this tendency showed little variation regardless of the sampling region (Fig. 5).

CONTENTS OF GAGS IN DRY CARTILAGE
The quantitative analysis of GAGs was carried out on 70 specimens of dry cartilage prepared from division of the femoral heads and the acetabula obtained from 10 hip joints by the method described previously. In accordance with the results of preliminary experiments the GAG fractionation was carried out on a resin column employing 0.3 M, 0.5 M, and 3 M NaCl solutions. Uronic acid in 0.5 M and 1.5 M fractions was determined as indices of hyaluronic acid and chondroitin sulphate respectively, and hexose in 3 M fraction was determined as keratan sulphate. The results of the analysis are shown in Figs 6, 7, and 8, in which the sugar contents were plotted on a graph against the sampling areas of the cartilage.

Fig. 5 Gel chromatography of GAGs in 3 M fractions on sephadex G-200. (A) Weight-bearing, femoral head. (B) Weight-bearing, acetabulum. (C) Nonweight-bearing, femoral head. (D) Nonweight-bearing, acetabulum. $\bullet$ — $\bullet$ Hexose; $\circ$ — $\circ$ Uronic acid.

Fig. 6 Uronic acid analysis of 0.5 M fractions (as index of hyaluronic acid).

Fig. 7 Uronic acid analysis of 3 M fractions (as index of chondroitin sulphate).

Fig. 8 Hexose analysis of 3 M fractions (as index of keratan sulphate).

Fig. 9 Molar ratio of uronic acid to hexose in 3 M fractions (as index of chondroitin sulphate/keratan sulphate).

Table 2 Analysis of uronic acid and hexose content of human hip joint cartilage (as μg per mg dry cartilage)

Fraction	Weight-bearing	Nonweight-bearing	Significant differences*
Acetabulum			
Uronic acid (0.5 M Fr)	1.53 ± 0.20	1.13 ± 0.34	$p < 0.005$
Uronic acid (3 M Fr)	21.0 ± 3.2	17.5 ± 4.6	$p < 0.01$
Hexose (3 M Fr)	22.7 ± 4.8	16.8 ± 6.8	$p < 0.005$
Femoral head			
Uronic acid (0.5 M Fr)	2.56 ± 0.50	2.12 ± 0.46	NS
Uronic acid (3 M Fr)	25.1 ± 0.50	14.1 ± 2.9	$p < 0.001$
Hexose (3 M Fr)	32.4 ± 5.7	11.2 ± 2.9	$p < 0.001$

Mean $\pm$ SD. Uronic acid as glucuronic acid, hexose as galactose. *Significant differences between weight-bearing and nonweight-bearing. NS=not significant. Fr=fraction.

The uronic acid content of 0.5 M fraction (i.e., hyaluronic acid) in the femoral head showed no significant differences between weight-bearing and nonweight-bearing regions, while in the acetabulum it was significantly higher in the weight-bearing portion. The ratio of uronic acid to hexose in 3 M fraction (i.e., the chondroitin sulphate/keratan sulphate) was significantly higher in the nonweight-bearing regions of both the femoral heads and the acetabula (Fig. 9). These results suggest that the increase of GAGs at the weight-bearing region is mainly due to a remarkable increase of keratan sulphate. All the results are summarised in Table 2.

The GAG content in the acetabulum was highest at the area 3 and tended to decrease toward the areas 1 and 5. A relatively large standard deviation was observed in the amounts of uronic acid and hexose at regions 1 and 5.

Discussion

A microfractionation method for the analysis of minute amount of GAGs was developed by authors

employing a high-porous anion-exchange resin, HPA-10, which had about a hundred times larger capacity for GAG than that of Dowex-1. This method is simpler than the excellent but complicated method of Antonopoulos *et al.*,¹⁸ and it requires a considerably smaller column size than that of Schiller *et al.*¹⁹

Galactosamine and uronic acid were found in almost equimolar amounts in 1 M and 1.5 M saline fractions, which indicate the presence of chondroitin sulphate in these fractions. The existence of keratan sulphate was also expected from the electrophoretic pattern of these fractions and from the considerable amounts of hexose detected along with glucosamine. However, the hexose content is too high for it to be explained as originating only from keratan sulphate. Both these experiments indicate the broad heterogeneity of keratan sulphate in the samples.

The GAG content of weight-loaded cartilage samples both from the femoral head and from the acetabulum was higher than that of nonloaded samples. Especially in the femoral head, both chondroitin

sulphate and keratan sulphate were extremely rich at weight-loaded areas. In the acetabulum all GAG members were rich in area 3, and they tended to decrease in the areas 1 and 5, but not so clearly as in the femoral head. These apparent differences in the distribution of GAGs in the acetabulum and the femoral head seem to have resulted from the following fact. In the femoral head weight-bearing and nonweight-bearing regions could be clearly separated, whereas in the acetabulum the 2 regions were continuous, with no clear-cut boundaries. The arbitrary content of GAGs at areas 1 and 5 in the acetabulum may depend on a relatively larger individual variation of morphological structure of the cartilage in these areas.

Maroudas *et al.*⁴ carried out a charge-density analysis on the GAGs in the perifoveal cartilage of the femoral head, and they concluded that there was negligible variation in GAG content from site to site. In contrast Venn⁹ reported in a similar study that the content of total GAGs was maximum on the superior surface and then gradually decreased towards the inferior region. In the case of the knee joint cartilage Ficat and Maroudas²⁰ indicated that there were no significant differences between the GAG content of lateral, medial, and central zones of the human patella cartilage. On the other hand Bjelle²¹ found a significantly higher content of both chondroitin sulphate and keratan sulphate, especially the latter, in cartilage from the caudal surface (subjected to maximal load) than from the posterior surface ('non-essential' cartilage).

Thus the results of these studies have differed with regard to the relation between GAG distribution and the loading regions. This might have been caused by differences in the procedures and the subjects used in the studies. The results of our study seem to be consistent with those of Venn⁹ and Bjelle.²¹

Some additional reports may support our results. Sweet *et al.*²² and Thonar *et al.*²³ found more hyaluronate and much more keratan sulphate in the articulating areas of maximum contact than in the minimum contact areas, and therefore they inferred that some keratan sulphate might be synthesised as a reaction to loading. Carterson *et al.*²⁴ showed that chondroitin sulphate significantly increased in the load-bearing cartilage in relation to loading stimulation. These results agree with the present observation that the weight-bearing cartilage contained higher amounts of every GAG.

The topographical distribution of GAGs in the cartilage should be considered with respect not only to loading conditions but also to the thickness of the cartilage,⁷⁻⁹ because the superficial and deeper portions of the cartilage contain different concentration of GAGs. The cartilage collected in the present study

seems to be thick in weight-bearing regions and thin in nonweight-bearing regions.^{25, 26} Therefore it is possible that such tangential distribution of GAGs might cause greater concentrations of them in the thicker cartilage.

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