A Proposed Structure of Chondroitin 6-Sulfate Proteoglycan of Human Normal and Adjacent Atherosclerotic Plaque

William D. Wagner, Brian J. Salisbury, and H. Alan Rowe

Chondroitin sulfate proteoglycan monomers were prepared from intima media minces of grossly normal human aorta and adjacent fatty fibrous atherosclerotic plaques. Glycosaminoglycan chains prepared from monomer from normal aorta displayed a normal distribution profile on Ultrogel ACA 54 with a $K_v$ of 0.48, whereas those of atherosclerotic aorta displayed a bimodal distribution (major peak, $K_v$ 0.35; minor peak, $K_v$ 0.70). The $M_v$ of glycosaminoglycans from normal aorta was estimated to be $1.5 \times 10^5$. For atherosclerotic aorta, the majority of chains were $2.0 \times 10^4$ while the smaller population was $1.2 \times 10^4$. All glycosaminoglycans were identified as chondroitin sulfate sulfated at the C-6 position. The amino acid compositions of both core proteins were similar with $M_v$ of about $1.6 \times 10^5$. After B-elimination in the presence of sodium borohydride prior to acid hydrolysis, chondroitin sulfate proteoglycan from normal aorta had reductions in serine from 105 to 68 and in threonine from 117 to 55. For the monomer from atherosclerotic plaque, reductions in serine and threonine, respectively, were from 103 to 81 and from 107 to 77 residues per 1000. The results suggested fewer chondroitin sulfate chains and oligosaccharides on the core protein in the proteoglycan of atherosclerotic plaque. Compared to normal aorta, substituted serines and threonines in the proteoglycan of atherosclerotic plaque were about half, respectively, 38% vs 21% for serine, 53% vs 28% for threonine. It is estimated that in atherosclerotic plaque there are fewer, but longer, chondroitin sulfate chains per core protein, translating into a smaller overall monomer size in atherosclerotic plaque. (Arteriosclerosis 6:407–417, July/August 1986)

During the last several years an expansion of studies on the structure of proteoglycans (PG) in different types of tissues has led to a further definition and better understanding of these complex carbohydrate macromolecules as tissue-specific and structurally discrete subunits or monomers. The exact function of specific PG located in intra-, peri-, and intercellular environments needs further definition. However, the following general functions have been discussed for these polyanionic components: space filling, maintenance of a compressive stiffness or resilience property of a tissue, maintenance of a viscoelastic property of a tissue, facilitation of polymer transport through matrix, regulation of solvent transfer from region to region within tissue, involvement in limiting diffusion of macromolecules through connective tissue, regulation of ion mobility, involvement in growth and calcification of collagen fibers, serving as transport vehicles for cell products, regulation of cell growth, and regulation of morphogenesis. Changes in the chemical and/or metabolic properties of PG have been reviewed for conditions such as arthritis and tumorogenesis. A recent review as well as earlier reviews provide comprehensive information on the structural features of PG in a variety of tissues and fluids.

One class of PG, chondroitin sulfate-containing PG (CSPG), has been reported in a variety of tissues. The structure of these PG monomers varies considerably. As an example, in bovine nasal cartilage the well-studied monomer consists of a core protein of $M_v 2.2 \times 10^6$ with about 100 covalently attached chondroitin sulfate chains, each with a $M_v$ of 15,000 to 20,000 and about 50 keratan sulfate chains, each with a $M_v$ of 5000 to 8000. The overall monomeric unit has a $M_v$ of $1.4 \times 10^7$. In contrast, the CSPG chondroitin 4-sulfate proteoglycan carrier of platelet factor 4 located in the α granules of platelets is a low $M_v$...
(59,000) monomer consisting of only four CS chains covalently linked to a core protein of about 10,000 M,12 Studies by Muir and Roden among others (see reference 21) have resulted in the identification of the covalent linkage site between the core protein and CS as the neutral trisaccharide sequence Gal β 1,3-Gal β 1,4-Xyl β 1-Ser. In tissues such as articular cartilage, the CS-PG monomers associate with hyaluronic acid through ionic interactions.2,20 This association maintains a high concentration of monomer in the extracellular matrix and functionally provides a viscoelastic matrix that serves to trap water when the tissue is subjected to a compressive load, thus retarding extensive tissue deformation.

In artery the major PG is CS-containing.27-31 If it functions primarily in the extracellular matrix as suggested by Iverius,32 it may have a role in regulating the flow of macromolecules such as plasma constituents in the normal artery. Should the structure, concentration, or physical properties such as binding to hyaluronic acid be modified, the consequences may include retention of cholesterol, perhaps through associations with plasma low density lipoproteins (LDL).

The involvement of PG in the retention of LDL within the artery wall with the subsequent development of atherosclerosis stems from several studies. It has been demonstrated that plasma LDL accumulates early in the development of atherosclerosis,33-36 and there is evidence that PG preferentially bind LDL rather than high density lipoprotein (HDL).36,37 Both glycosaminoglycans (GAG) and LDL accumulate early in the atherosclerotic lesion,36,38 and GAG-LDL complexes have been isolated from fatty streaks and atherosclerotic plaques.40,41 Finally, it has been reported that LDL-artery PG complexes made in vitro are internalized by macrophages.42

With the progression of atherosclerosis it has been firmly established that the content of arterial sulfated GAG increases.38,39 However, no information is available on the structure of the CS-PG monomer in the atherosclerotic plaque. This study was designed to further our previous reports on human and pigeon aortic PG by providing additional and detailed chemical information on CS-PG monomers, and to describe the structural features of the monomer isolated from the human atherosclerotic plaque. The objectives were to describe the structure and composition of the CS-PG isolated from human normal aorta and to determine if this PG is structurally distinct in adjacent areas of aorta with atherosclerosis.

Methods

Chemicals and Reagents

Ultrapure GdnHCl was purchased from Schwartz/Mann (Orangeburg, New York) and used without further purification as the PG extraction solvent, GdnHCl (98% minimum) for chromatographic purposes was obtained from Eastman Organic Chemicals (Eastman Kodak Company, Rochester, New York). Alcian blue (98% minimum), m-hydroxydiphenyl, benzamidine HCl, 6-aminohexanoic acid, and hexadecyl-pyridinium chloride (CPC) were also obtained from Eastman Kodak Company, Rochester, New York. CPC was purified by two recrystallizations from aceton-water. Papain (22 U/mg 2X crystalized), D-glucuronic acid (Grade I), D(+)-glucosamine-HCl, D(+)-galactosamine HCl, bovine serum albumin (fraction V), tyramine hydrochloride, and phenanthroline were obtained from Sigma Chemical Company (St. Louis, Missouri). Sodium 125I-iodine (mCi/µg) was obtained from Amersham Corporation (Arlington Heights, Illinois). Diallo membranes were purchased from Amicon, (Danvers, Massachusetts); Sepharose CL 4B and DEAE Sepacel from Pharmacia Fine Chemicals, Pharmacia, Incorporated, (Piscataway, New Jersey); Ultrogel AcA 54, LBK Instruments, Incorporated, (Rockville, Maryland); Chondroitin 4-sulfate, chondroitin 6-sulfate, hyaluronidase (strepococal), chondroitin ABC lyase, and chondroitin AC lyase were purchased from Miles Laboratories, Incorporated (Elkhart, Indiana). Hyaluronic acid, heparan sulfate, dermatan sulfate, chondroitin 4-sulfate, and chondroitin 6-sulfate preparations were NIH standards obtained from Drs. M. B. Mathews and J. A. Cifonelli of the University of Chicago (Contract NO1-AM-5-2205 from the National Institute of Arthritis, Metabolism, and Digestive Diseases). All other chemicals and solvents used were reagent grade and were obtained from Fisher Scientific Company (Fairlawn, New Jersey).

Analytical Procedure

Hexuronic acid was measured according to the procedure of Blumenkrantz and Asboe-Hansen44 with D-glucuronic acid used as the standard. The total hexosamine was assayed by the method of Blumenkrantz and Asboe-Hansen45 with galactosamine HCl used as the standard. Protein was measured according to the method of Lowry et al.46 with bovine serum albumin used as the standard. Chondroitin 4-sulfate and chondroitin 6-sulfate concentrations were determined by analyzing the unsaturated disaccharide concentrations.47

Amino acid analyses were done on samples hydrolyzed in 6 N HCl at 110° C for 18 hours. Hydrolysates were evaporated to dryness under nitrogen and dissolved in 1.0 ml distilled water. Amino acid analyses were done with a Technicon Sequential Multisample Amino Acid Analyzer (Technicon Instruments, Tarrytown, New York) according to the method of Benson and Hare.48 Nortesine was used as an internal standard, and each amino acid value was corrected for color formation. Proline was determined by the method of Troll and Lindsley.49

Aortic Preparation

Thoracic and abdominal aortas were obtained from the autopsy service, Department of Pathology, the Bowman Gray School of Medicine of Wake Forest University. For the study, subjects comprising as narrow an age range as possible were selected to minimize differences due to the postmortem times and individuals with documented clinical histories of any connective tissue disorder, chronic hypertension, chronic obstructive pulmonary disease, or diabetes mell-
For this study we used the aortas of five subjects with a mean age of 51 years (range 40 to 60) in whom the primary cause of death was attributed to gastrointestinal hemorrhage, acute myocardial infarction, pulmonary thromboembolus, thermal burns, or unknown factors. The post-mortem interval, defined as the time elapsed between death and freezing of the prepared tissues, ranged from 5.5 to 8 hours, with a mean of 6.7 hours. Throughout the preparation of samples, all aortas were kept chilled on ice. The adventitia and outer media were removed by using a common plane of dissection through the media. The resulting preparation contained mainly intima with a consistent thickness of inner media throughout the aorta. Grossly normal areas were separated from areas of raised atherosclerotic plaques. The atherosclerotic plaques were characterized as raised fatty fibrous lesions without complications such as hemorrhage, ulceration, or extensive mineralization. In a number of aortas, small areas, that were fatty streaks or that contained lesions with varying degrees of complications were not included. Approximately the same percentage of normal aorta or atherosclerotic plaque was obtained from each of the individual aortas selected to comprise the tissue pool. The preparations of intima media were minced into 2 x 3 mm pieces, were weighed and frozen in distilled water at -20° C.

**Extraction and Isolation of Proteoglycan Types**

Aortic minces were thawed and extracted with 4.0 M guanidine HCl in 50 mM sodium acetate, pH 4.5 (15 ml/g wet tissue), containing 10 mM disodium EDTA, 0.1 M 6-aminohexanoic acid, 5 mM benzamidine hydrochloride, 3 mM phenanthroline, and 5 mM tryptamine hydrochloride (Figure 1). The extract was filtered through Whatman filter paper, and the extracted tissue was rinsed once with 4.0 M GdnHCl. Rinses were added to the original extract. PG not extracted with GdnHCl were isolated as GAG following proteolytic treatment of the tissue as described.

The GdnHCl solution containing the PG was concentrated in an Amicon ultrafiltration cell fitted with a PM-30 Diaflo membrane and chromatographed on Sepharose CL-4B by using 4.0 M GdnHCl, 0.05 M sodium acetate (pH 5.8) as the eluant. Chromatographic columns were 1.4 x 90 cm and were pumped at a flow rate of 15 ml/h. Selected fractions were analyzed for hexuronic acid.

**Purification of Chondroitin Sulfate Proteoglycans**

The artery PG fractions containing CS eluting near the column void volume were pooled, concentrated (PM-30 Diaflo membrane), and further purified by isopycnic gradi-

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**Figure 1.** An outline of the procedures used to isolate specific arterial proteoglycan monomers.
ent centrifugation in cesium chloride (p density 1.40 g/ml) in 4.0 M GdnHCl. The samples were centrifuged at 90,000 g for 50 hours at 15°C in a Beckman Model L8 ultracentrifuge with a Beckman type 65 rotor. Following centrifugation, the bottom 2/5 of the gradient was dialyzed against 7.0 M urea in 0.05 M Tris-HCl (pH 6.8) and applied to an 1.5 x 15 cm columns of DEAE-Sepharose. PG were fractionated by an initial elution of 75 ml of 7.0 M urea in 0.05 M Tris-HCl (pH 6.8) followed by batches of urea containing NaCl concentrations ranging from 0.15 to 2.0 M. Fractions were dialyzed exhaustively against water at 4°C, were lyophilized, and then dissolved in 0.05 M sodium acetate (pH 5.8) for subsequent analyses.

**Electrophoretic Separation of Glycosaminoglycans**

The electrophoretic procedure used to separate GAG on cellulose acetate has been described in detail. The buffer was 0.3 M calcium acetate (pH 10.0), and samples were electrophoresed at 8 mA for 3 hours. GAG were stained with 0.5% Alcian blue (pH 5.8), and the cellulose acetate strips were cleared and fixed as described.

**Enzymatic Degradation of Glycosaminoglycans**

Chondroitin ABC lyase (Proteus vulgaris, Miles Laboratories) and chondroitin AC lyase (Arthrobacter aurescens, Miles Laboratories) digests were conducted as described by Oike et al. Briefly, 1 μmol of proteoglycan (as hexuronic acid) was incubated with 1.0 ml of the enzyme digestion mixture containing either chondroitin ABC lyase (1.5 units) or chondroitin AC lyase (1.0 units). Controls containing proteoglycan and the digestion mixture without enzyme were included. The enzymatic reactions were carried out at 37°C for 40 minutes, and the reactions were terminated by reduction and carboxymethylation.

**Proteoglycan Iodination**

Core proteins were iodinated by the chloramine-T method described by Greenwood et al. PG was dialyzed against 0.25 M sodium phosphate (pH 7.5), and 3.5 μg (as hexuronic acid) was iodinated with 0.7 mCi Na125I, 50 μg chloramine-T, 120 μg sodium metabisulfite, and 1.7 mg sodium iodide. Free Na125I was removed by using P-6-DG Biogel (Bio-Rad, Richmond, California) with 0.25 M sodium phosphate (pH 7.5) as the column buffer.

**Determination of Proteoglycan Core Protein Molecular Weight**

Core protein molecular weights were determined on preparations after chondroitinase ABC lyase treatment and chromatography on calibrated columns. A HPLC system consisting of an Altex 110 A pump, a model 153 UV detector, and a model 210 sample injection valve (Beckman Instruments, Incorporated, Irvine, California) was used. Two different HPLC column systems were used for the molecular weight determinations: a TSK G4000 SW column (600 mm x 7.5 mm, pore size 13 μm, Beckman Instruments, Incorporated, Irvine, California) and an OH-1000 + OH 500 (both 250 mm x 4.6 mm, 10 μm pore size, Brownlee Labs, Santa Clara, California) that were linked in series. Both systems were calibrated with protein molecular weight standards in the aqueous column buffer. The OH-1000 + OH-500 system was also calibrated by using polystyrene standards (Supelco, Incorporated, Bethlehem, Pennsylvania) in chloroform. The mobile phase for the TSK G4000 SW system was 4.0 M GdnHCl, 0.05 M sodium acetate (pH 5.8). The mobile phase for the OH-1000 + OH-500 HPLC column system was 0.1 M Tris-PO4 (pH 7.6). For both systems the flow rate was 0.5 ml/minute, and 0.25 ml fractions were collected. Radioactivity was measured with a Gamma 4000 solid scintillation counter (Beckman Instruments, Incorporated).

**Results**

From 50 g of grossly normal intima media and 28 g of atherosclerotic tissue we obtained 28.7 mg and 13.5 mg of hexuronic acid, respectively, containing material following extraction with 4.0 M GdnHCl. PG extraction efficiency estimated from measurements of hexuronic acid on the extract and the GAG hexuronic acid released from the GdnHCl treated tissue was 70% for the normal aorta preparation and 79% for the preparation of atherosclerotic aorta. From previous studies on human and pigeon aorta, chondroitin sulfate is the predominant GAG of the aorta and a major component of the 4.0 M GdnHCl extract. Other GAG present in the GdnHCl include hyaluronic acid, heparan sulfate, and dermatan sulfate. The GAG remaining within the tissue after treatment with GdnHCl were not studied further but have been reported to be mainly heparan sulfate and about 25% to 30% of the aortic chondroitin sulfate.

The GdnHCl extracts, which included essentially all of the sample except material removed for hexuronic acid measurement, were concentrated and chromatographed on Sepharose CL 4B by using 4.0 M GdnHCl, 0.05 M sodium acetate, (pH 5.8) as the eluant. Two major peaks of hexuronic acid-positive material were detected in both aortic preparations (Figure 2). One peak comprised about 65% of the total hexuronic acid and was localized near the column void volume while the other peak eluted at a Kav of 0.36 for normal aorta and 0.37 for atherosclerotic aorta. From our studies of human and pigeon aortas, we determined that chondroitin sulfate was the main component of Peak 1 material and that dermatan sulfate was a major component of Peak 2 material. For each sample, material from the column void (Kav 0–0.24) was pooled, concentrated, and further purified on a CsCl density gradient.

After centrifugation, each gradient contained a thin floating pellicle containing 1% to 3% of the total gradient hexuronic acid. For normal and atherosclerotic aorta, 8.3 mg and 2.6 mg of hexuronic acid, respectively, was recovered from the bottom two-fifths of the density gradients (d ≤ 1.45 g/ml). For both PG samples, this fraction as 75% of the total hexuronic acid of the gradient. PG from the bottom of the density gradients were subjected to exchange dialysis with 7.0 M urea, 0.05 M Tris-HCl (pH 5.8) applied to DEAE-Sepharose. After elution, the preparations were dialyzed, lyophilized, and dissolved in 6
ml of 0.5 M sodium acetate (pH 5.8). All subsequent analyses were sampled from this preparation.

The hexuronic acid distribution pattern for both samples of PG were similar (Table 1). About 80% of the PG in both samples eluted with 0.50 M NaCl, the same ionic strength at which a standard preparation of chondroitin 6-sulfate eluted. Lesser but significant amounts of PG were eluted with 0.25 M and 0.35 M NaCl. For any given salt fraction, GAG and protein percentages of PG from the normal and the atherosclerotic aorta were similar. There were differences, however, between salt fractions; the PG in the 0.35 M fraction had a greater percentage that was protein (35%...
to 39%) compared to the 0.25 M and 0.50 M fraction with protein percentages of 25% to 28%. Hexosamine/hexuronic acid molar ratios determined for the 0.5 M fraction of the chondroitin 6-sulfate standard and the 0.25 M fraction of hyaluronic acid were 0.91 and 1.18, respectively. GAG and protein percent composition of PG in each fraction. The GAG value used in calculating these percentages are not considered.

### Table 1. Composition of Human Aortic Proteoglycans Eluted from DEAE-Sephacel

<table>
<thead>
<tr>
<th>NaCl fraction (M)</th>
<th>Hexuronic acid (μg)</th>
<th>Total hexosamine (μg)</th>
<th>Protein (μg)</th>
<th>Hexosamine/hexuronic acid ratio</th>
<th>GAG (%)</th>
<th>Protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal aorta</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>254 (11%)</td>
<td>272 (8%)</td>
<td>244 (10%)</td>
<td>1.16</td>
<td>73</td>
<td>27</td>
</tr>
<tr>
<td>0.35</td>
<td>269 (11%)</td>
<td>494 (15%)</td>
<td>372 (15%)</td>
<td>1.99</td>
<td>85</td>
<td>35</td>
</tr>
<tr>
<td>0.50</td>
<td>1899 (78%)</td>
<td>2577 (75%)</td>
<td>1916 (75%)</td>
<td>1.47</td>
<td>72</td>
<td>28</td>
</tr>
<tr>
<td>Atherosclerotic aorta</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>107 (6%)</td>
<td>104 (6%)</td>
<td>100 (6%)</td>
<td>1.05</td>
<td>74</td>
<td>26</td>
</tr>
<tr>
<td>0.35</td>
<td>160 (9%)</td>
<td>194 (12%)</td>
<td>272 (15%)</td>
<td>1.32</td>
<td>61</td>
<td>39</td>
</tr>
<tr>
<td>0.50</td>
<td>1423 (85%)</td>
<td>1358 (82%)</td>
<td>1382 (79%)</td>
<td>1.03</td>
<td>73</td>
<td>27</td>
</tr>
</tbody>
</table>

The percentages within parentheses indicate the distribution among the three fractions for each sample. For known standard GAG preparations, 82% and 18% of the hyaluronic acid eluted at 0.25 M and 0.35 M NaCl, respectively, while 100% of the chondroitin 6-sulfate eluted at 0.5 M NaCl. Hexosamine/hexuronic acid molar ratios determined for the 0.5 M fraction of the chondroitin 6-sulfate standard and the 0.25 M fraction of hyaluronic acid were 0.91 and 1.18, respectively.

GAG and protein percent composition of PG in each fraction. The GAG value used in calculating these percentages were estimated by dividing the hexuronic acid values by 0.38. These values for percent composition are only estimates, since xylose and galactose, components of the GAG linkage region, and other non-GAG oligosaccharides are not considered.

### Table 2. Carbohydrate Composition of Chondroitin Sulfate Chains of Proteoglycan

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Normal aorta</th>
<th>Atherosclerotic plaque</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total disaccharides</td>
<td>29.0 μg</td>
<td>20.7 μg</td>
</tr>
<tr>
<td>Δ di 6-S</td>
<td>29.2</td>
<td>21.3 μg</td>
</tr>
<tr>
<td>Δ di 4-S</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total hexuronic acid</td>
<td>24.0 μg</td>
<td>18.4 μg</td>
</tr>
<tr>
<td>Δ di 6-S/uronate</td>
<td>1.20</td>
<td>1.18</td>
</tr>
</tbody>
</table>
A. Gel chromatography of glycosaminoglycans (GAG) chains prepared from proteoglycans of normal (X—X) and atherosclerotic aorta (•—•). For each preparation, a sample of the fraction eluted from a DEAE-SephaCell column with 0.5 M NaCl was treated with papain and the GAG was isolated after precipitation with hexadecylpyridinium chloride (CPC). Isolated GAG were chromatographed through a 1.4 x 90 cm column of Ultrogel AcA 54 eluted with 0.1 M sodium acetate (pH 5.8).

B. Separation of individual glycosaminoglycans (GAG) on cellulose acetate. Samples for electrophoresis were obtained from fractions indicated by the bars above Figure 2 A. Electrophoresis was done by using 0.3 M calcium acetate (pH 10.0) for 3 hours at 8 mA. HA = hyaluronic acid, HS = heparan sulfate, DS = dermatan sulfate, CS = chondroitin sulfate. Sample 1 = GAG of proteoglycans (PG) isolated from normal aorta. Samples 2 and 3 = GAG of PG isolated from adjacent atherosclerotic plaques.

Does not remove any O- or N-linked oligosaccharides, the molecular weights determined can reflect an overestimate of size, depending upon the oligosaccharide composition. With the calibrated TSK 4000 SW column, chromatography of iodinated core protein preparations showed one broad peak of radioactivity. Similar results were obtained with the OH 100 and OH 500 column. Estimates of molecular weights for both PG preparations were about 1.6 x 10^5 (Figure 4).

The amino acid compositions of the two preparations of PG are given in Table 3. The proteins contained high levels of glutamic acid, threonine, serine, aspartic acid, and glycine. With the possible exceptions of glutamic acid, proline, isoleucine, and tyrosine, both preparations of core protein were similar in amino acid composition and were comparable to the core proteins prepared from the CS-PG of bovine aorta. Additional samples of the PG preparations were subjected to B-elimination in the presence of sodium.
Figure 4. Proteoglycans from normal aorta (A) and from atherosclerotic plaque (B) were radiolabeled, treated with chondroitase ABC lyase, and chromatographed on a TSK G4000 SW column with 4.0 M GdnHCl, 0.05 M sodium acetate (pH 5.8) used as the mobile phase. The insert shows the $K_v$ of several molecular weight standards. a = thyroglobulin, $M_r = 670,000$; b = $\gamma$-globulin, $M_r = 158,000$; c = ovalbumin, $M_r = 44,000$; d = myoglobin, $M_r = 17,000$; e = cyanocobalamin, $M_r = 1,350$.

Table 3. Amino Acid Composition (Residues/1000 Residues) of Core Protein of Human Aortic Chondroitan Sulfate-Containing Proteoglycans

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Normal aorta (NA)</th>
<th>Atherosclerotic plaque (PQ)</th>
<th>Bovine aortic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>87</td>
<td>89</td>
<td>92</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>117</td>
<td>107</td>
<td>93</td>
</tr>
<tr>
<td>Threonine</td>
<td>109</td>
<td>103</td>
<td>104</td>
</tr>
<tr>
<td>Serine</td>
<td>208</td>
<td>176</td>
<td>150</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>101</td>
<td>60</td>
<td>47</td>
</tr>
<tr>
<td>Proline</td>
<td>80</td>
<td>82</td>
<td>95</td>
</tr>
<tr>
<td>Glycine</td>
<td>73</td>
<td>88</td>
<td>71</td>
</tr>
<tr>
<td>Alanine</td>
<td>76</td>
<td>80</td>
<td>62</td>
</tr>
<tr>
<td>Valine</td>
<td>&lt;10</td>
<td>87</td>
<td>66</td>
</tr>
<tr>
<td>Cysteine</td>
<td>35</td>
<td>39</td>
<td>75</td>
</tr>
<tr>
<td>Methionine</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>n.d.</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>29</td>
<td>11</td>
<td>23</td>
</tr>
<tr>
<td>Leucine</td>
<td>37</td>
<td>37</td>
<td>75</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>34</td>
<td>34</td>
<td>37</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>34</td>
<td>34</td>
<td>20</td>
</tr>
<tr>
<td>Lysine</td>
<td>34</td>
<td>34</td>
<td>28</td>
</tr>
<tr>
<td>Histidine</td>
<td>34</td>
<td>34</td>
<td>28</td>
</tr>
<tr>
<td>Arginine</td>
<td>34</td>
<td>34</td>
<td>28</td>
</tr>
</tbody>
</table>
| Protein: NA = 262 µg; PQ = 190 µg sum of amino acid residues: NA = 198 µg; PQ = 229 µg

*Data taken from Table II, PGA, of reference 31.
†Data taken from Table III, PG-50, of reference 52.

decreased from 107 to 77. These results suggest fewer GAG and oligosaccharides associated with the core protein in PG of atherosclerotic plaque. Compared to normal aorta, substituted serine and threonine in PG of atherosclerotic plaque were about half (serine) and 53% vs 28% (threonine), respectively.

To propose a model for the possible structure of the two PG monomers we have assumed that the CS is linked via a seryl linkage and that all serines were substituted only with CS. If this is the case, based upon the size of the core protein, the PG monomer from the atherosclerotic plaque would have only 26 CS chains/core compared to 39 for the PG from normal aorta. The results of the compositional data are summarized in Table 4. Based upon the data and the assumptions indicated, the structures of the two PG differ primarily in the carbohydrate moieties. The results suggest that PG monomers from the atherosclerotic plaque have fewer CS chains per core protein, resulting in a reduced overall monomer size ($6.8 \times 10^5$) compared to that of normal aorta ($7.5 \times 10^5$).

**Discussion**

Although there are several reports on the isolation and characterization of artery PG, many of these earlier studies were concerned with the examination of the GAG moiety and most were reporting mixed types of PG monomers or isolated GAG chains from a mixed population of PG. Three
Table 4. Comparison of Chondroitin 6-Sulfate Proteoglycan Monomers Isolated from Normal Human Aorta and Adjacent Atherosclerotic Plaque

<table>
<thead>
<tr>
<th>Monomer</th>
<th>Normal Aorta</th>
<th>Athero-sclerotic Plaque</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chondroitin sulfate chains/core protein Mr</td>
<td>15,000</td>
<td>20,000</td>
</tr>
<tr>
<td>Core protein Mr</td>
<td>1.6 x 10^5</td>
<td>1.6 x 10^5</td>
</tr>
<tr>
<td>Substituted serine</td>
<td>38%</td>
<td>21%</td>
</tr>
<tr>
<td>Substituted threonines</td>
<td>53%</td>
<td>28%</td>
</tr>
<tr>
<td>Chondroitin sulfate chains/core protein (based upon the number of glycosylated serines)</td>
<td>39</td>
<td>26</td>
</tr>
<tr>
<td>Core protein Mr</td>
<td>1.6 x 10^5</td>
<td>1.6 x 10^5</td>
</tr>
<tr>
<td>Estimated monomer size</td>
<td>7.5 x 10^5</td>
<td>6.8 x 10^5</td>
</tr>
</tbody>
</table>

Number in parentheses indicates minor component.

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The monomer molecular mass assessed by Schmidt et al.¹³ after chromatography on a Sepharose 2B column calibrated with reference PG was reported to be only 130,000, whereas the CS-DS-PG described by Oegema et al. had a Mr of 1.5-2.0 x 10⁶ as determined by sedimentation equilibrium centrifugation. This latter size is more in line with our estimate of 7.5 x 10⁵ for the human PG in the present study. In our earlier reports,²⁷,²⁸ we indicated that the DS-PG is a discrete monomer from the CS-PG. We are currently investigating the detailed structure of human artery DS-PG. Our preliminary results suggest that it has a core protein molecular weight of about 50,000, similar to that described for DS-PG of pigeon aorta.⁴ The DS-PG monomer in general is easily discriminated from the CS-PG by its smaller hydrodynamic size, by the presence of iduronic acid as a component of the GAG, by a different GAG chain migration on cellulose acetate, by a smaller core protein molecular weight and different amino acid composition, by an inability to aggregate with hyaluronic acid, and by greater synthetic rate in pigeon artery organ culture systems.²⁷,²⁸,⁵³,⁵⁴ Although there are several reports on the changes in the GAG content and concentration in arteries with various degrees of atherosclerosis and of changes in the distributions of individual GAG as atherosclerosis progresses,²⁷,²⁸,⁵³,⁵⁴ the significance of the present report is the demonstration of a structural or qualitative change in the CS-PG monomer in the atherosclerotic plaque. These structural changes reflect an average change in the conformation of the entire population of CS-PG monomers. Since we presented evidence that the core proteins were not different in size, it is possible that the CS chains of the monomer isolated from atherosclerotic plaque are spaced further apart. Our assumption is that GAG are evenly spaced on both core proteins. Although this has not been demonstrated for artery CS-PG, reports for cartilage PG suggest that CS is evenly spaced in the CS-rich region of the core.²³ If we assume that plasma LDL might have easier access to sulfate and carboxyl groups within GAG chains that are spaced wider apart on a core protein compared to those GAG chains more closely packed, then monomers from the atherosclerotic plaque, which would have fewer and longer CS chains, might have a greater potential to bind and retain LDL within the intercellular space. It may then be possible for such a conformational change in the PG to be responsible for increasing the rate at which atherosclerosis develops, perhaps through continued and increased binding to insudated plasma LDL.

In the present study we attempted to reconstruct the chondroitin sulfate PG monomer through the studies of the component parts. Of these, the estimates of the number of CS chains per core protein should only be taken as an upper limit since we have estimated the number of the CS chains, assuming that all serine are substituted with glycosaminoglycan chains. To date there is no information on the O-linked or N-linked oligosaccharides thought to exist as a component of the CS-PG. The report by Chang et al.,⁵³ described the structure of a chondroitin sulfate PG synthesized by aortic smooth cell in culture. In this study, O and N-linked oligosaccharides were a significant component of
the PG monomers. If the CS-PG is similar to the CS-PG produced by artery-derived smooth muscle cells in culture, then we would expect that, assuming a similar core protein molecular weight, the CS-PG may average as low as 20 GAG chains per core (normal aorta) and as low as 10 chains per core (atherosclerotic). These calculations are based on the ratio 1:6 for galactosamine of the O-linked oligosaccharides to galactosamine in the CS chain. Even if we have overestimated the number of CS chains per core of protein, there still remains a large difference in the number of substituted serine or threonine residues on the two core proteins. Even though the one peak of iodinated core protein was obtained for both preparations of normal and atherosclerotic aorta, the peak was broad, suggesting the heterogeneity in structure also seen by others after electrophoresis on polyacrylamide.

We should also note that our estimates for core protein size are average upper limits of size since the preparations were prepared following chondroitinase ABC treatment of the PG and contain the trisaccharide GAG linkage region as well as any oligosaccharides. There is an additional noteworthy point for comparison in structure. There appears to be a consistent difference in the size of the GAG chains comparing products of smooth muscle cells in culture and CS-PG isolated from arterial tissue; the former have chains of molecular weights (~43,000) while the latter have molecular weights (~15,000). In our completed studies on the isolated CS-PG of pigeon aorta smooth muscle cells, we have also determined that CS chains are of large molecular weights (~45,000) (data not presented).

The results of the present study illustrate that the CS-PG monomer from atherosclerotic plaque is structurally distinct from the PG monomer in the adjacent normal artery. There is a possibility that these monomers are either different gene products or have been modified after synthesis or have been modified through proteases that are abundant in atherosclerotic tissue. The fact that amino acid compositions and molecular weights of the isolated core proteins of the two preparations were similar does not support an argument of distinct gene products resulting either from modifications in the genetic expression of the existing arterial smooth muscle cells or from the existence of distinctive cell types within the atherosclerotic plaque. Based upon the differences seen in CS chains and substituted threonines, it appears that the monomers may have been modified through changes in post-translational processing.

Whether the distinct structure of the CS-PG in the atherosclerotic plaque involves any alteration in normal function remains a question. In other studies, we have assessed the sedimentation coefficients of additional preparations of chondroitin sulfate PG from normal human aorta and adjacent atherosclerotic plaque. In those studies the patients' ages and the PG preparation were similar to those in this report. Sedimentation velocity measurements indicated a smaller CS-PG monomer in plaque (S0.1 = 5.3) vs normal aorta (S0.1 = 8.7). One can propose that the smaller, conformationally distinct PG monomer may be related to increased binding and retention of LDL in the vessel wall. We have presented some evidence that the CS-PG monomers isolated from atherosclerotic plaque have a reduced capacity to associate with hyaluronic acid to form high molecular weight aggregates. Perhaps the existence of such monomers within the matrix in an unordered structure may make possible their association with other components of the plasma, such as LDL. If, as described by Salisbury et al., uptake of these complexes by macrophages occurs, an early atherosclerotic lesion may ensue.

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A proposed structure of chondroitin 6-sulfate proteoglycan of human normal and adjacent atherosclerotic plaque.

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